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DIMORPHIC QUEENS IN AN AMERICAN ANT (LASIUS LATIPES WALSH).[1]

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On the afternoon of September 17 of the current year the senior author had occasion to witness the nuptial flights of several species of *Lasius* in an open wood near Rockford, Illinois. These flights occurred almost simultaneously from mound nests of *Lasius niger* var. *americanus*, *L. claviger* and *L. latipes*. The first species is ubiquitous in all open country in the Northern States, especially where the soil is sandy or loamy. Owing to the dingy color of the workers, males and females, and the relatively small size of the colonies, the nuptial flight of this species offers nothing of special interest or beauty. It is quite otherwise with some of the yellow *Lasius*, of which at least eight species are known to occur in the United States, namely: *L. aphidicola* Walsh, *speculiventris* Emery, *brevicornis* Emery, *myops* Forel, *interjectus* Mayr, *claviger* Roger, *latipes* Walsh, and *Murphyi* Forel. The last is known only from North Carolina and Colorado. *L. interjectus*, *aphidicola*, *claviger* and *latipes* build large mound nests, often a foot or more in diameter and several inches high, either in open grassy places or about the bases of rotting stumps. These mounds are shot through with living grass and covered with little openings for the ingress and egress of the ants. *L. latipes* in some localities prefers to build its nests under rather large stones. This is the case at Colebrook, Connecticut, for example. Unlike *L. niger* and its varieties the yellow species of the genus appear to be nocturnal in their habits and *L. myops* largely subterranean. At any rate the workers of these various species are not seen to leave the nests in the day-time except during the nuptial flight of the males and virgin females.

[1] Contributions from the Zoölogical Laboratory of the University of Texas. No. 46.

This flight, especially in the case of *L. latipes*, presents a beautiful spectacle. At the moment when the great swarming

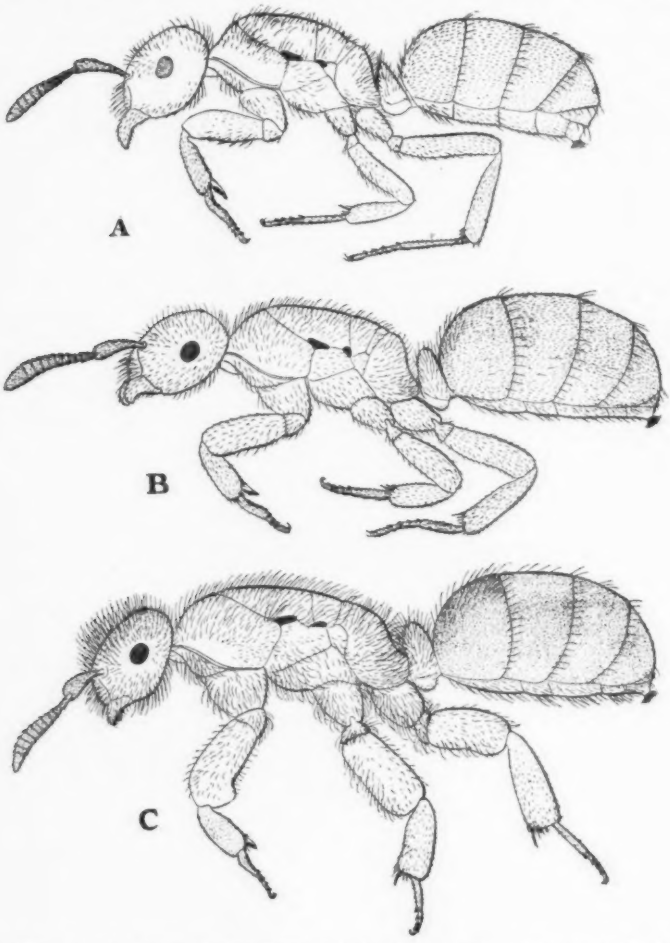


FIG. 1. *A*, *Lasius claviger* Roger ♀; *B*, *Lasius latipes* Walsh *a*-♀; *C*, *L. latipes β*-♀. The wings are omitted.

impulse seizes the colony, the shining amber-yellow workers, together with the hosts of sable males and large tawny-red females, break in a flood from the main openings of the nest.

The winged forms hasten up the slender grass-blades on which they rock for a few moments, while filling their tracheæ with the pure air of the upper world, then one by one spread their glittering wings and soar into the air like sparks rising from a fire.

While watching a colony during this interesting culmination of its annual development, the senior author noticed females of two different kinds issuing in numbers from the same openings of the grass-covered mound. The majority of these females were the remarkably pilose individuals, of a rich fulvous red, with extremely broad and flat legs and abnormally short, feeble tarsi, which have always been regarded as the true females of *L. latipes*. Among these, however, there were several hundred females which were perceptibly smaller, of a deep brown color, much less pilose, with only moderately broadened and compressed legs and with much longer tarsi. Both forms mingled with the workers and males and took flight together within the same half hour. Although the unusual character of this observation was fully appreciated at the time, circumstances made it impossible to excavate the nest and search its penetralia for the mothers of these very different virgin females. It seemed best to leave the nest for careful study at some future time and to collect a large number of the workers, males and females at the surface.

In this paper we will designate as the β-female the highly aberrant form (Fig. 1, *C*) with the excessively flattened legs, *i. e.*, the form which has hitherto passed as the true and only female of *latipes;* the other (Fig. 1, *B*) we will call the α-female. These designations will suffice for present purposes and will leave the facts uncolored by the conjectural meaning of this singular dimorphism.

A few days after the above recorded observations were made the senior author returned to Texas, and soon afterwards, with the aid of the junior author, undertook an examination of all the material of *L. latipes* collected during three consecutive summers in three different localities. This was easily possible because the specimens from different nests had been kept by themselves in separate vials of alcohol. There were, in all, collections from ten separate nests, as recorded with the date of capture and the personnel of each colony in the following table:

Nest No. 1. Woods Hole, Mass., Aug., 1900. ☿, ♂, β-♀.

Nest No. 2. Woods Hole, Mass., Aug., 1900. ☿, β-♀.

Nest No. 3. Colebrook, Conn., Aug. 19, 1901. ☿, β-☿.
Nest No. 4. Colebrook, Conn., July 21, 1901. ☿, β-☿.
Nest No. 5. Colebrook, Conn., Aug., 1901. ☿, ♂, α-☿, β-☿.
Nest No. 6. Colebrook, Conn., Aug. 12, 1901. ☿, ♂, α-☿.
Nest No. 7. Rockford, Ill., July 6, 1902. ☿.
Nest No. 8. Rockford, Ill., Aug. 15, 1902. ☿, ♂.
Nest No. 9. Rockford, Ill., Aug. 20, 1902. ☿, ♂.
Nest No. 10. Rockford, Ill., Sept. 17, 1902. ☿, ♂, α-☿, β-☿.

Nest No. 1 was collected by Miss Adele M. Fielde. No. 2 was an artificial nest seen in one of the laboratories at Woods Hole. It contained many workers and a single deälated female of the β-type. Of the ten nests in the table it will be seen that three contained workers only, or workers and males. These may be disregarded as having no bearing on the subject under consideration. Of the seven nests containing females, four contained β-females only; two contained both α- and β-females, and one contained α-females only. Thus it is seen that the α-female, which has not been observed hitherto, occurred in three out of seven nests, and in two very widely separated localities. This is significant in view of the fact that *L. latipes* is not nearly so common a species as *L. claviger*, *interjectus*, *myops* or *aphidicola*.

Examination of a considerable number of females of both types failed to disclose any forms intermediate in structure or pilosity. In nest No. 5, however, all the β-females had the same deep brown color as the α-females of the same nest. There were often considerable individual variations in the venation of the wings, but these variations occurred in both types indiscriminately. The types were not connected by intermediate forms and were indistinguishable from each other by any characters in the shape, color, or venation of the wings, so that these organs could be omitted in the figures and in the comparative statements to be given below.

A study of the descriptions of *L. latipes* by previous writers shows very clearly that the β-female has played a very important rôle in the recognition of the species, because it differs so markedly in structure and pilosity from the females of any of the known members of the genus. The original description by Walsh (**'62,** p. 311) is so brief and inadequate that it would have been con-

	L. claviger. ♀*	L. latipes. α-♀	L. latipes. β-♀
Length of thorax	2,517	3,390	3,487
Length of gaster	3,055	3,681	3,487
Length of petiole	285	303	392
Height of petiole	872	926	943
Length of antennal scape	926	890	854
Apical breadth of antennal scape	178	230	267
Length of funiculus	1,566	1,513	1,370
Breadth of funiculus	214	231	249
Length of fore femur	1,264	1,264	1,335
Length of middle femur	1.193	1,175	1,282
Length of hind femur	1,356	1,388	1,460
Breadth of fore femur	427	498	659
Breadth of middle femur	303	427	605
Breadth of hind femur	356	463	623
Length of fore tibia	1,068	1,015	979
Length of middle tibia	1,015	1,050	1,086
Length of hind tibia	1,513	1,513	1,442
Breadth of fore tibia	320	356	445
Breadth of middle tibia	267	338	427
Breadth of hind tibia	267	356	481
Length of fore spur (strigil)	338	320	231
Length of middle spur	231	249	249
Length of hind spur	267	267	267
Length of whole fore tarsus	1,015	905	729
Length of first fore tarsal joint	570	516	374
Length of second fore tarsal joint	89	89	89
Length of third fore tarsal joint	89	71	71
Length of fourth fore tarsal joint	89	71	53
Length of fifth fore tarsal joint	178	160	142
Length of whole middle tarsus	1,175	961	818
Length of first middle tarsal joint	605	534	409
Length of second middle tarsal joint	125	107	89
Length of third middle tarsal joint	107	89	89
Length of fourth middle tarsal joint	107	89	71
Length of fifth middle tarsal joint	231	142	160
Length of whole hind tarsus	1,691	1,406	997
Length of first hind tarsal joint	979	783	570
Length of second hind tarsal joint	196	160	107
Length of third hind tarsal joint	142	125	89
Length of fourth hind tarsal joint	107	89	71
Length of fifth hind tarsal joint	267	249	160

signed to the limbo of useless specific diagnoses except for the mention of the extraordinarily flattened legs in the female, a character which is, moreover, emphasized in the specific name. Walsh had only two specimens of the β-female. The locality of the types is not given, but was probably Rock Island, Illinois. It was the flattening of the legs of *L. claviger*, a trait still more pronounced in *latipes* and visible also in *interjectus* and the more recently discovered *Murphyi* that led Mayr ('62, p. 51) to separate these forms as the genus *Acanthomyops*. Later he reduced

* The dimensions are in micra.

this genus to subgeneric rank under *Lasius*, where it is still used to include those species which have 3- instead of 6-jointed maxillary palpi. Mayr redescribed (**'66,** p. 889) the β-female from a defective specimen from Wisconsin, and says that he was at first tempted to place it in a new genus on account of its remarkable appearance. That he refrained from doing this is evidence of his keen taxonomic insight. Later writers, like Emery (**'93,** p. 638), have included the β-female in the table of *Lasius* species as distinguishable from all other females by having "the hind tarsus shorter than the much flattened tibia." The discovery of the α-female, which has the hind tarsus longer and the tibia much less dilated, makes it more difficult to recognize the species. This has induced us to make a closer study of *L. latipes* and of the allied *claviger* in all the sexual phases.

Comparison shows that the α-female is almost intermediate between the β-female and the female of *claviger*. This is clearly shown in the figures, in the table of measurements on p. 153, drawn up by the junior author, and in the two-column statement of the principal differences between the α- and β-females, as compared with the female of *L. claviger* as a standard:

α-female of Lasius latipes.	**β-female of Lasius latipes.**
1. Dark brown, like *L. claviger* ♀.	1. Fulvous red, in one nest (No. 5) dark brown like the α-female.
2. A little more pilose and pubescent than *L. claviger* ♀.	2. Much more pilose and pubescent.
3. A little larger.	3. Considerably larger and longer.
4. Thorax longer in proportion to the gaster.	4. Thorax much longer in proportion to the gaster.
5. Mesonotum and scutellum as in *claviger* ♀.	5. Mesonotum and scutellum flatter.
6. Petiole thicker, higher and more rounded above than in *claviger* ♀.	6. Petiole considerably thicker, higher and more rounded above.
7. Mandibles similar to those of *claviger* ♀.	7. Mandibles with fewer teeth than in *claviger* ♀ (Fig. 3*C*).

8. Antennal scape and funiculus shorter and broader. (Fig. 2, *B*.)

8. Antennal scape and funiculus still shorter and broader. (Fig. 2, *C*).

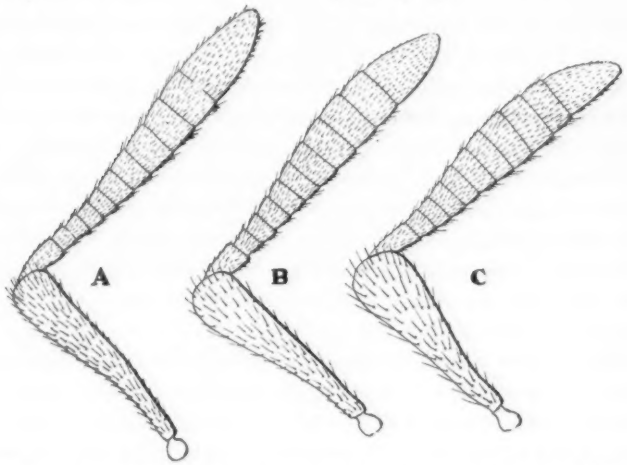


FIG. 2. *A*, Antenna of *Lasius claviger* ♀ ; *B*, of *L. latipes* α-♀ ; *C*, of β-♀.

9. Trochanters, femora and tibiæ broader and more flattened.

9. These joints extraordinarily flattened and dilated.

10. Strigil a little smaller.

10. Strigil much smaller.

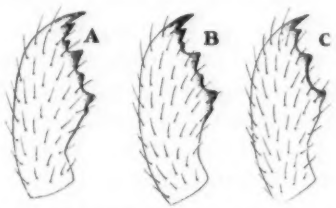


FIG. 3. *A*, Mandible of *Lasius claviger* ♀ ; *B*, of *L. latipes* α-♀ ; *C*, of β-♀.

11. All the tarsi a little more tapering.

11. Tarsi rapidly tapering.

12. Middle and hind tarsi nearly as long as the tibiæ.

12. Middle and hind tarsi much shorter than the tibiæ.

Turning now to a comparison of the two species, *latipes* and *claviger*, as exhibited by the workers and males, we find but few

points of difference, so that we are compelled to regard the two species as very closely related to each other. The worker *latipes* has a thicker petiole, which is distinctly blunt and rounded above, and the hairs are somewhat more abundant and evenly distributed on the dorsal surface of the gaster. In the worker *claviger*, on the other hand, the petiole is thinner anteroposteriorly and sharply cuneate above when seen in profile, and the gaster is less uniformly hairy and somewhat more shining. The males of the two species differ much as do the workers in the shape of the petiole. Moreover the male *claviger* is decidedly larger, more robust and blacker than the male of *latipes*. These differences, especially in the males, are easily appreciated in the living specimens when they are seen in numbers, but are necessarily more obscure in dried cabinet specimens.

From these comparatively slight differences between the males and workers of the two species we should naturally expect to find a corresponding similarity in the females. It is quite obvious that the α-female is the very form which satisfies this requirement, whereas the β-female presents extreme characters which make it appear like a decided sport or aberration from the normal type of *Lasius* female. It would seem, therefore, that the β-female is the one for which we are most in need of an explanation, although it is connected with the females of normal form by a rather complete series of gradations, *i. e.*, through the females of the following species, beginning with the most extreme form: *L. Murphyi*, α-female of *latipes*, *claviger*, *interjectus*. The remarkable configuration of the legs and antennæ, the color and pilosity of the β-female all suggest some peculiarity of habit or habitat the nature of which remains to be determined by further observation and experiment.

We come now to the important question: What is the meaning of this dimorphism in the females of *L. latipes?* From the fragmentary data at our command it would seem that four different hypotheses might be advanced to explain this peculiar phenomenon:

1. It may be suggested that the α- and β-females really belong to two distinct species. According to this view the α-female might be regarded as the true queen of *latipes*, whereas the

β-form would represent the queen of some inquiline or symbiotic species. Although this explanation is readily suggested by the well-known cases of dulosis and xenobiosis in ants, we are, nevertheless, bound to reject it for the following reasons: Though the β-females were taken in several nests and, in one case, were seen to celebrate their nuptial flight at the very same time as the α-females, no males or workers which could represent any species except *latipes* were to be found in the nests. The same argument would hold *mutatis mutandis*, were we to consider the β-form as the only true female of *latipes*. The workers and males of all the known North American Lasii have been accounted for, and there is still a female form left over, so that there is no species known that could be enslaved by, or live as an inquiline with, *L. latipes*. We should have to suppose that the inquiline species was represented by females only, and this is most improbable. Finally, the deep coloration above noted as occurring in the β-females of nest No. 5 would indicate that both the α- and β-females belong to the same species. We believe, therefore, that this hypothesis may be safely rejected.

2. It may be suggested that the α-female is the normal female of *latipes*, whereas the β-females are diseased forms — individuals afflicted with some strange emmet elephantiasis or acromegaly! But even apart from the very frequent occurrence and uniform development of the β-females, dissection shows that such a view cannot be seriously entertained. Their internal structure is in no respect abnormal. The fat body is well developed and the ovaries are in the same stage and have the same normal structure as the ovaries of the α-females. If anything, the β-females are more vigorous, somewhat larger and supplied with more fatty tissue (even in the distal lobes of the large fore femora!) than the α-females. In a word, the β-females are somewhat above normal, while the α-females, so far as we are able to judge, are quite normal. Hence this hypothesis, also, may be safely rejected.

3. The dimorphism may be regarded as the result of hybridism between *L. claviger* and *L. latipes*. This view is supported by the following considerations:

(*a*) Both species occur in the very same localities, and *latipes* is much rarer than *claviger*. Hence the queens of the latter may

find cross-fertilization by males of their own species from other nests very difficult and fertilization by males of *claviger* a relatively easy matter.

(*b*) The nuptial flights of the two species may occur simultaneously. In fact, the senior author witnessed a flight of *claviger* from a nest not twenty feet away from the *latipes* nest and at the very same time (3.30 P. M.) as the above-described flight of the latter species. And it may also be stated that both these nests were large and must therefore have existed side by side for some years. We could suppose that a β-female of *latipes* in some previous year had been fertilized during her nuptial flight by a male *claviger* and had returned into the parental nest to give birth to the α-females which celebrated their nuptial flight on the 17th of September, 1902.

(*c*) This view is also supported by the fact that the α-female is so clearly intermediate in nearly all its characters between the female *claviger* and the β-female, as has been shown in the above tables.

The arguments that can be brought to bear against the hypothesis are the following :

(*a*) We have failed to find any hybrid workers in the nests containing the α- and β-females. This should be the case unless we suppose that all the hybridized β-females produced only queens.[1] But it must be borne in mind that the hybrid between the worker *claviger* and worker *latipes* would differ presumably from the parent species only in intermediate pilosity and in having a petiole intermediate in shape. Such differences would not be easily detected, as anybody will confess who has examined a large series of workers of the two species. The workers are of small size and the petiole is sometimes decidedly variable even within the limits of the same species of *Lasius*.

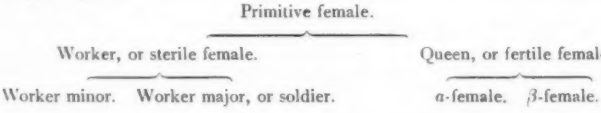
(*b*) It is improbable that hybridization could occur so frequently in a state of nature as appears to be indicated by the high percentage of nests containing α-females, and their occurrence in such widely separated localities. If we are really confronted by a case of hybridism we are almost compelled to believe

[1] Obviously the male offspring of the hybridized queen would not be affected, since they arise from unfertilized eggs.

that the *α*-female must be sterile, notwithstanding her well-developed ovaries, or the two species would long since have merged into one.[1]

(*c*) It seems improbable that such an aberrant creature as the *β*-female would mate with the male of another species, but this argument loses much of its force when we stop to reflect that the *claviger* male is very similar to the *latipes* male even in the structure of its genitalia.[2]

4. We may suppose that we are dealing with a true case of dimorphism in the female sex. On first thought this seems improbable because dimorphic queens, in the strict sense of the term, are unknown among ants. But when we stop to consider that the social bees and wasps exhibit an essentially similar dimorphism, except that one of the two winged forms, the worker, is sterile (and this may also be the case with the *α*-female of *L. latipes!*) there is nothing preposterous in this view. Moreover, in ants the wingless workers have themselves in many species become dimorphic, developing soldier and typical worker forms, either perfectly distinct from each other or connected by a series of intermediates. Why, then, may we not expect the winged queens in some cases to exhibit dimorphism among themselves, especially when dimorphism "runs in the blood," so to speak, of all the social Hymenoptera? And why may not *L. latipes* be such a species in which the old and deeply-rooted tendency is breaking out in a novel form? This would at least complete the theoretical possibilities in female ants as represented in the folfowing diagram:



It thus appears that of the four hypotheses, two may be rejected as too improbable to be entertained, and that the true meaning of

[1] Unless, indeed, the Mendelian law be supposed to operate with unprecedented clearness in this particular case.

[2] In this connection, however, it is interesting to note that Marchal ('96, p. 45) failed to induce mating between two very closely allied species of wasp (*Vespa germanica* and *V. vulgaris*).

the dimorphism of the females of *L. latipes* is to be sought in the direction of hybridism or of dimorphism *sensu stricto*. Only further observation and especially experiment can enable us to decide which of these interesting alternatives confronts us.[1]

For the present we incline to the belief that the α- and β-females of *latipes* represent true dimorphic forms, and see in this condition an interesting repetition of what may have led to the differentiation of the primitive winged female ant into workers and queens. It is granted on all sides that insects like the ants, social wasps and bees, which present three sexual phases, viz., males, queens and workers, are to be derived from forms with only a single female form. In the bees and wasps there can be no question that this original female form was winged like the male, and we should expect this to be the case also with the ants, but so eminent a myrmecologist as Professor Emery takes quite a different view of the matter ('95*b*, p. 775). He says: "If the above considered derivation of ants from Mutillid-like Hymenoptera be granted, we must suppose, furthermore, that in primitive ants, as in the Mutillids, the males were winged, but the females wingless, and that the latter subsequently reacquired wings. This supposition is upheld by the fact that wingless females are most commonly met with among the Dorylinæ and Ponerinæ, *i. e.*, in those very groups of ants which are the most primitive, more rarely among the Myrmicinæ, and most rarely, and, so far as I am aware, only as individual anomalies, in the Dolichoderinæ and Camponotinæ. The frequency of occurrence of wingless females is, therefore, inversely as the phyletic stage of development of the different groups of ants.

[1] In the Lepidoptera and Hymenoptera, if we except a few cases like the Torymid Chalcididæ, the female sex seems to be more prone to dimorphism than the male. In Diptera the few recorded cases of dimorphism occur in males; *e. g.*, in the Brazilian *Curupira torrentium* (Fritz Mueller, '81; Osten Sacken, '95) and the North American Syrphid *Mallota cimbiciformis* (Williston, '86). Among the Coleoptera *Dytiscus* presents dimorphism in the females, while some of the Anthribidæ are said to show it in the males. The dimorphism seen in the "high" and "low" males of the Scarabæidæ among the Coleoptera and the "high" and "low" male Dermaptera (*Forficula auricularia*) observed by Bateson ('94, pp. 40–42), resembles that of the female *Lasius latipes* in being a normal and excess development of the individuals of the same sex. In the latter case, however, the two forms are not connected by intermediate variations.

"Not only is the normal occurrence of wingless females among existing species evidence of a similar condition among the primitive ants, but it also furnishes the most natural explanation of the origin of the wingless workers. I surmise that *the ancestral ants constituted small societies of wingless females, among which sterile individuals were subsequently differentiated as workers.* The wings, so readily deciduous in the queens of existing ants, were newly acquired from rudiments still persisting in the ontogeny, by a process of reversion to the winged ancestors."

We are unable to assent to this view, for the following reasons:

1. While there is no end of evidence to show that the most diverse insects have lost their wings during phylogeny, there is not, to our knowledge, a single insect which can be satisfactorily shown to have reacquired these organs. At any rate the losing of wings is a much easier process than their acquisition.[1]

Emery's hypothesis postulates a winged condition in both sexes of the ancestors of Mutillidæ, a loss of the wings in the females of the Multillid-like ancestors of ants, a persistence of this primitive condition by inheritance in the ancestral Formicidæ and a comparatively recent reacquisition of wings in the females of all except the Dorylinæ and the few Ponerine genera which have wingless females (*Leptogenys*, *Acanthostichus*). This would seem to be a needless complication of matters, apart from the fact that it is venturesome to invoke the obscure principle of reversion to account for the reacquisition of organs.

2. Existing wasps and bees certainly show the possibility of differentition into workers and queens prior to the loss of wings.

[1] This is an interesting case of a principle to which Headley ('01, pp. 100, 101) has recently called attention: "The sudden loss of horns brings out a point to which, I think, attention has never been directed in discussions on pammixis. The evolution of new characters is a gradual process requiring ages of time. Geology shows that the stag's antlers have grown step by step from small beginnings. But they might be completely lost in a single generation. The horns of cattle, though less magnificent, are none the less the slow product of ages of unintermitted selection. But by a sudden freak they disappear utterly in an individual here and there, or leave only a dangling vestige attached to the skin.

"Those evolutionists who love symmetrical theories, mapped out regardless of observed facts, imagine a process of retrogression by which all the stages are retraced in ordered succession. What actually happens is usually very different. An elaborate organ is suddenly much reduced and mutilated or suddenly disappears altogether."

And there is no strong evidence to show that this condition did not exist in the ancestral ants, for the Dorylinæ are hardly in the direct line of Formicid descent, and the Ponerinæ, though very primitive, still show the differentiation into winged queens and wingless workers in some of their most generalized genera (*Cerapachys*, *Sysphincta*, *Proceratium*, etc.).

3. That the most natural way of accounting for the wingless workers is through loss of the organs of flight in one of the two winged female forms, is also indicated by the phenomena of ergatomorphism among male ants. It is known that in a few sporadic species belonging to several genera the males are wingless and have assumed a worker-like form, especially in the development of the thorax. These species are, *Anergates atratulus* (Schenck '52), *Formicoxenus nitidulus* (Adlerz '84), *Cardiocondyla Stambuloffii* (Forel '92), *Ponera punctatissima* (Emery '95*a*) and *P. ergatandria* (Forel '93). This same reduction of the wings is shown in a more or less advanced condition in some male Mutillidae. All these cases are most naturally explained by loss of the organs of flight, and we are justified in adopting the same explanation to account for the wingless condition of the workers. Our view of the matter, therefore, would differ from Emery's in assuming that in the ancestors of the ants all three forms, workers, queens and males, were alike winged, and that the workers lost their wings either suddenly in accordance with Headley's principle, or concomitantly with the atrophy of the ovaries and the assumption of the other worker characters. Thus it would be the workers that have lost their wings and the queens have not reacquired, but retained these organs which came to them as the common heritage of all the Pterygote insects.

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FUSION OF BLASTOMERES AND NUCLEAR DIVISION WITHOUT CELL-DIVISION IN SOLUTIONS OF NON-ELECTROLYTES.

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I. INTRODUCTORY.

Blastomeres derived from the cleavage of a single egg-cell have not infrequently been observed to reunite or flow together under various abnormal conditions, producing an apparent reversal of the normal cleavage-process. Thus Dreisch[1] in 1893 observed a refusion of blastomeres in the 4-cell stage of *Echinus* as a result of exposure to temperatures higher than the normal (26 degrees). In 1894 Graf[2] found that compression of *Arbacia* eggs in early stages (16 to 32 cells) also led to a fusion of adjacent cells and even in several instances to a complete reversion from one cleavage stage to the stage immediately preceding (*e. g.*, from 16 cells to 8 cells). In 1896 J. Loeb[3] observed similar phenomena in *Ctenolabrus* eggs deprived of oxygen; he ascribed the effect to a liquefaction of the cell-membrane and a consequent flowing together of the protoplasm of adjacent cells. Phenomena of a somewhat different though closely related order have also been observed from time to time. Ziegler,[4] while studying the cleavage of enucleated blastomeres, observed that these cells frequently underwent only partial division, the cleavage-furrow forming and subsequently disappearing; and similar "abortive attempts at division" have been described by other authors.[5] In such instances the cleavage-process appears at first to proceed normally, but it remains incomplete and the cell resumes its original form when the impulse to division ceases.

[1] Driesch, *Zeitschrift für wissenschaftliche Zoölogie*, 55, 1893, p. 10.
[2] Graf, *Zoölogischer Anzeiger*, 17, 1894, p. 424.
[3] J. Loeb, *Archiv für die gesammte Physiologie*, 62, 1896, p 249.
[4] Ziegler, *Archiv für Entwicklungsmechanik*, 6, 1898, p. 282.
[5] Cf. Wilson, *Archiv für Entwicklungsmechanik*, 12, 1901, p. 529.

Both of the above classes of phenomena present the following general resemblances: the surface-area of the entire egg first of all undergoes a marked increase; increase of area is accompanied by corresponding alterations in the spherical form of the egg; an increase of surface-extent is thus normal to cleavage and indeed may with justification be regarded as one of its most essential features.[1] The increased surface-area does not in the above instances remain unaltered, or increase still further as normally, but undergoes a secondary and abnormal decrease, seen in the smoothing of the incipient cleavage-furrows or the refusion of the already separate blastomeres. These alterations in surface-area are almost undoubtedly to be ascribed to alterations in the surface-tension of the egg. The primary increase of surface seen in cleavage thus indicates a general lowering of the normal surface-tension; this in the above instances appears to be followed by an increase of surface-tension which leads to the succeeding regressive series of changes.

The problem of cleavage thus resolves itself, partially at least if not entirely, into the problem of the nature of the conditions producing alterations of surface-tension at certain definite regions of the egg-surface. It is believed that the experiments about to be described throw light upon this problem. They have demonstrated that cytoplasmic cleavage is prevented in solutions of non-electrolytes, although nuclear division continues; and also that a strong tendency to fusion makes its appearance in the blastomeres of eggs transferred to such solutions in early cleavage-stages. Since under these conditions the electrolytes normally present in the egg must diffuse outward into the surrounding medium, it is to be inferred that cleavage is closely dependent upon the presence of electrolytes within the egg-protoplasm. The manner in which ions derived from the dissociation of the contained electrolytes may conceivably effect alterations in the surface-tension of the egg will be considered in some detail below.

II. Experimental.

The following experiments were performed during the past summer at Wood's Holl as part of a series on which I was then

[1] Compare Rhumbler: Merkel und Bonnet's Ergebnisse, 8, 1898, p. 605 et seq.

engaged with the aim of determining the influence of the electric current on cell-division in the eggs of *Asterias* and *Arbacia*. Solutions of low conductivity (non-electrolyte solutions with a trace of sea water and isotonic with the latter) were used to insure the passage of the current *through* the eggs. The results of this investigation are incomplete and their publication is deferred for the present. It was found, however, that the simple action of the solutions upon the eggs presented certain interesting peculiarities which form the subject of the following description.

The solutions used were molecular solutions (which are approximately isotonic with sea-water) of urea, glycerine, and cane-sugar, especially urea, which has the least injurious action on the eggs.

In these solutions neither starfish nor sea-urchin eggs are capable of cleavage. Eggs transferred to m-urea-solution shortly after fertilization remain living for several hours and nuclear division continues, although more slowly than under normal conditions. No complete cleavages occur, although many eggs, especially those of *Asterias*, assume irregular or amœboid forms. In *Arbacia* a partial constriction may appear at the equator of the egg if the transfer is made shortly before the time of the first cleavage, but in the majority of instances no such signs of incipient cleavage appear.

The following is a record of a typical experiment:

July 12. *Arbacia* eggs, fertilized at 10:27 A. M., were washed with m-urea and transferred to 100 c.c. of the same solution at 11:10 A. M. At 11:35 the control eggs in the sea-water were beginning to segment; in the urea-solutions no signs of segmentation were visible, but a few eggs had become somewhat amœboid in outline. At 11:55 a larger proportion of urea-eggs were amœboid; a certain number were dumb-bell-shaped with a clear area (nucleus) in each enlargement, the division into two halves remaining incomplete. The great majority of eggs showed no such signs of incipient division. At this time the control eggs were in the 2- and 4-cell stages.

Urea-eggs of this series were preserved in picro-acetic and sublimate-acetic fixing fluids,[1] at stages corresponding to the 2-, 4-, 8- and 32-cell of the control. Subsequent examination of stained preparations showed that nuclear division had proceeded in the urea-solutions although cytoplasmic division had been en-

[1] Boveri's picro-acetic and saturated aqueous mercuric chloride with 2 per cent. glacial acetic acid.

tirely prevented, as above described. In other words, the withdrawal of the electrolytes from the egg in some way prevents the transmission of the division-impulse from the nucleus (which apparently initiates the same) to the body of the cell.

A similar effect has been observed by J. Loeb[1] and Norman[2] to follow the withdrawal of water from the egg by means of sea-water concentrated by the addition of sodium chloride or magnesium chloride; other instances of nuclear division without cell division have been recorded by various authors (Boveri, Chabry, Driesch, Roux).[3] The fact that this phenomenon is seen in solutions of non-electrolytes is of peculiar interest as indicating the importance of the part played in cell-division by the ions present in the cytoplasm. Nuclear division, on the other hand, is apparently independent of the presence of such ions.

Not only is cleavage prevented by transfer to non-electrolyte solutions, but a strong tendency to fusion manifests itself in the blastomeres of eggs transferred during early cleavage stages. This fusion takes place most readily with starfish eggs and in solutions of urea; glycerine- and sugar-solutions are relatively injurious to the eggs and are accordingly less favorable.

The following record will illustrate:

June 27, 1902. Starfish eggs, fertilized at 12:25 P. M., were transferred at 2:15 P. M. while in the 2-, 4-, and 8-cell stages to m-urea-solution. On examination at 3:30 the blastomeres were found in many instances to be completely re-fused, the eggs having apparently reverted to the original unicellular condition. Such eggs form rounded masses of protoplasm each containing several nuclei (which do not fuse) and exhibiting in many cases irregular amœboid projections.

With eggs of more advanced stages (16- and 32-cell) fusion is rarely so complete as in earlier stages. Adjacent blastomeres, however, gradually flow together, and the resulting compound blastomeres, at first hour-glass-shaped, tend to round off, although slowly and as a rule incompletely. Fig. 1 is a representation of an egg with partially fused adjacent blastomeres of the 8-cell

[1] Loeb, J. *Archiv für Entwicklungsmechanik*, Bd. 2, 1896, p. 298.

[2] Norman, *ibid.*, Bd. 3, 1897, p. 106.

[3] For a summary of the observations of these authors cf. Korschelt and Heider, Vergleichende Entwicklungsgeschichte, allgemeiner Theil, erste Lieferung, Jena, 1902, p. 215.

stage. This egg presents an unusually regular appearance; typically the fusions follow no definite order and from the 8-cell stage complexes are more frequently obtained that consist of three, four or five more or less rounded and in part compound blastomeres of unequal size, the entire group remaining enclosed within the egg membrane. Contiguity seems to be the chief condition that determines which of the blastomeres undergo fusion; there is no indication that sister blastomeres reunite more readiiy than cells of different parentage; the fusions take place also after different intervals and with varying degrees of completeness. Thus a strict reversal of cleavage in the sense that the egg reverts to the stage immediately preceding (from 16 to 8 cells, etc.) is of exceptional occurrence. It is of interest to note that fusion is typically preceded by the assumption of a perfectly rounded or spherical form by each cell. The flattening at the surfaces of apposition is thus replaced by a curvature indicative of increased surface-tension (Fig. 2).

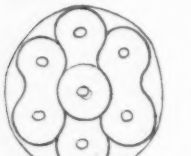


FIG. 1. Example of regular fusion of blastomeres of 8-celled stage. Urea solution. (*Asterias*).

FIG. 2. Rounding of blastomeres in fusion-product of 4-celled stage. Urea-solution (*Asterias*).

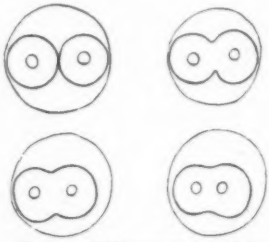


FIG. 3. Different stages of fusion of 2-cell stage in m-glycerine solution (*Asterias*).

In m-glycerine solutions fusions also occur, although in relatively fewer instances. Glycerine is specifically injurious to a far higher degree than urea, and swelling and granular disintegration early appear in many eggs. Different stages of fusion of blastomeres of the 2-cell stage are represented in Fig. 3. Frequently the separate blastomeres merely become rounded without undergoing fusion.

In m-cane-sugar solutions the tendency towards fusion is even less, in consequence apparently of the extreme viscidity and density of the solution and its tendency to withdraw water from the

egg. Actual fusion does not occur in this solution; the cells become perfectly spherical, touching only at points of their surfaces — indicating an increase of surface-tension — but without fusing.

In sea-urchin eggs similar phenomena are observed. In general fusion takes place more slowly and less completely in these eggs than in those of *Asterias*. *Arbacia* eggs placed in m-urea solution during the 2- and 4-cell stages exhibit fusion in a large proportion of instances; within thirty minutes after transfer all stages ranging through various dumb-bell-shaped compounds to completely rounded fusion-products are usually seen. Fusions occur also in later stages (16- and 32-cells) in the same manner as in *Asterias*. The blastomeres exhibit a similar tendency to the adoption of a spherical shape before fusion, indicating a preliminary increase of surface-tension.

Fusion occurs also in m-glycerine solutions; separate blastomeres gradually assume the rounded form, and in a few instances they fuse, but fusion is slower and less complete than in urea-solutions. The glycerine acts destructively on these eggs as on those of *Asterias*, leading before long to a swelling and granular disintegration.

III. Theoretical.

From the above experiments the conclusion may be drawn that cleavage, as well as the maintenance of the cleaved condition, is dependent upon the presence of electrolytes in the cytoplasm. The activity of these electrolytes must be regarded as due to the ions into which they dissociate. The exact nature of the rôle played by the ions is at present largely a matter of conjecture; but it is reasonable to infer that here, as in other relations, they act chiefly by virtue of the electrical charges which they carry. If this is admitted the following possibilities present themselves for consideration.

If the essential feature of cleavage is an enlargement of the egg-surface due to diminution of surface-tension over certain areas, the possibility at once suggests itself that this alteration of tension may be dependent upon certain known electrical influences,—to be more explicit, upon the appearance of a difference of electrical potential at the surface of contact between the egg

and the surrounding medium. That the surface-tension between two adjacent immiscible fluids may be so altered has long been known; the researches of Lippmann and Helmholtz have shown that under these conditions the surface-tension is greatest when the potential-difference of the two adjoining phases is zero, and decreases in definite proportion as the potential-difference increases.[1] The mutual repulsion characteristic of electrical charges of like sign produces at the surface of contact a certain expansive tension which opposes the normal surface-tension and diminishes the latter by that degree. The deformation of the charged mercury-drop, the expansion of the charged soap-bubble, are familiar phenomena dependent upon diminution of surface-tension so occasioned. It is thus possible that the surface-enlargement leading to cleavage may be a special instance of the Lippmann-phenomenon; if this is true we see at once the importance of the electrolytes, since the production of a difference of electrical potential between the two adjoining aqueous media (egg and sea water, separated by a semi-permeable membrane) can be accomplished only by a migration of ions.

These ions must, under the conditions, be situated within the egg; the appearance of a charge at the surface of the latter must therefore signify the gathering of a surplus of ions of one sign in the superficial regions of the egg-protoplasm. This implies the presence of a corresponding surplus of oppositely charged ions in the interior of the egg. Hence the presence of a surplus of ions of either sign in the peripheral region must imply the existence of a difference of electrical potential between the surface and the interior of the egg; and if the conditions of cleavage are as we have supposed, evidence of such potential-difference should be found at the time of cleavage. Is there any evidence of the existence of such difference of potential, and, if so, how is the same to be accounted for?

In answer to the first question it may be said at once that such evidence is by no means lacking, though of an indirect kind and hitherto variously interpreted. It is seen, in my opinion, in the typical appearances presented by the astral radiations and

[1] Cf. Ostwald, "Lehrbuch der allgemeinen Chemie," 2d Edition, Vol. II., pp. 920–948.

spindle-fibers of dividing cells. The resemblance of the lines of the mitotic figure to the electrical and magnetic lines of force has, needless to say, long been the subject of frequent comment. Now we know that if such an electrical potential-difference between exterior and interior exists, a condition of electrical strain or tension must also exist, the direction of whose lines of traction must correspond with that of the electrical lines of force. It has also been shown that the direction of the fibrils of coagulum in fixed preparations of albumin-solutions subjected during fixation to mechanical strain coincides closely with the direction of this strain (cf. Hardy,[1] Fischer[2]). We may therefore infer from the direction of the fibrils in fixed preparations of dividing cells that a condition of strain or tension exists between interior and periphery of the cell during mitosis, similar to that which would exist in the presence of an electrical potential-difference of the kind imagined above. This agreement confirms the theory that such potential-difference does actually exist. The tendency of the alveoli in echinoderm eggs to dispose themselves along corresponding lines is similarly to be interpreted as an instance of the tendency possessed by polarizable particles in an electric field to arrange themselves in rows along the lines of force.[3]

It may fairly be claimed therefore that it is no mere assumption to suppose that during cleavage the surface of the egg is charged differently from the interior. If such a surface-charge is present it will infallibly produce a lowering of the surface-tension with corresponding changes in the form of the egg. Cleavage, on the present theory, is the result of such changes: its conditions will be considered below in further detail.

If we grant then that during mitosis the surface of the egg is charged differently from the interior the question arises: how is this difference of potential established and maintained? In other words, what influence directs ions of one sign toward the periphery, the others toward the interior of the egg? This question ap-

[1] Hardy, *Journal of Physiology*, 24, 1899, p. 158.

[2] Fischer, Fixierung, Färbung und Bau des Protoplasmas, Jena, 1899.

[3] For experiments bearing directly on this question, see Gallardo, "Interpretacion Dinamica de la division Cellular," Buenos Ayres, 1902. Reviewed by M. Hartog in *Nature*, Vol. 67, 1902, p. 42.

pears at present susceptible of only partial reply. It is to be noted however that a marked potential-difference *invariably* makes its appearance (as evidenced by the appearance of astral radiations) whenever the nuclear chromatin passes into the highly condensed and chromatic phase, characterized by a high proportion of nucleic acid, which forms perhaps the most typical and constant of the peculiarities of mitosis. Since, chemically considered, this change denotes an increase in the acidity of the chromatic colloids, and since such acidity implies that the colloidal particles are negatively charged,[1] the inference has seemed reasonable that the inequality of distribution of the ions in the cytoplasm is due to the acquisition of a negative charge of high potential by the colloids composing the nuclear chromatin. I have elsewhere[2] called attention to this possibility and have pointed out that the spiral form of the chromatic filament in the prophase, and also the disposition, mode of division, and movements of the chromosomes, are strongly suggestive of the action of electrostatically charged bodies. If the chromatin carries a negative charge the anions will tend to approach the periphery of the egg as a result of the inductive action of this charge, while the kations will be attracted toward the chromatin. The result will be the establishment of a potential-difference within the cytoplasm of the kind indicated above, with a negative charge at the surface of the egg.

There exists experimental evidence of an apparently conclusive kind that the ions of opposite sign present in a solution may be separated by the inductive action of an electrostatically charged body. The original proof of this proposition appears to be furnished by the well-known and frequently-quoted experiment of Ostwald and Nernst.[3] Recently J. Olsen[4] has described experiments which lead to similar conclusions. This author has found that an electrolyte-solution is affected by an electrostatic charge in such a manner that the liquid nearest the charge assumes a charge of opposite sign to that of the charged body employed,

[1] Picton and Linder, *Journal of the Chemical Society*, 1897, LXXI., p. 568. Hardy, *Journal of Physiology*, 24, 1899, p. 288. See also Bredig, "Anorganische Fermente," Leipzig, 1901, p. 15.

[2] *American Journal of Physiology*, 8, 1903, p. 273.

[3] *Zeitschrift für physikalische Chemie*, 3, 1889, p. 120.

[4] *American Journal of Science*, 1902, Vol. XIV., No. 82.

while the remotest portion of the liquid assumes a charge of the same sign as that of the charged body. The liquid, in other words, behaves as if it contained charged bodies free to move (ions) which may be separated by the inductive action of an electrostatic charge, ions of opposite sign moving toward, those of the same sign away from the inducing charge.

In the cell the charged body on the above theory is represented by the chromatin; by the inductive action of its negative charge the anions are repelled toward the periphery of the egg while the kations are attracted toward the nucleus. The center of the astral radiations must on this view represent the region of highest positive potential — the region, that is, in which the kations are most densely aggregated. Exactly why there should be *two* such regions, and why these are situated on opposite sides of the nucleus and at some distance from the surface of the latter are questions of considerable difficulty which cannot be considered in detail in the present paper. It is evident that the conditions of electrostatic equilibrium in such a structure as the cell must be of somewhat complex nature. Later I hope to consider these and other questions in fuller detail; for the present their treatment is deferred.[1]

[1] I may suggest here briefly that the *form* of the cell must influence the distribution of the surface charges; and that it is for this reason that the direction of the spindle-axis bears certain well-defined relations to the principal axis of the cell; these relations have long been known and are formulated in Hertwig's laws of cell-division. If the cell possesses approximately the shape of a prolate ellipsoid — a not infrequent condition — it is to be expected that the repellent action of the internal negative charge will produce a tendency toward aggregation of anions at those regions of the surface-area which are most remote from the central charged body, *i. e.*, which adjoin the long axis of the cell. If the surface negative charge so induced attains sufficient density in these regions there must result a tendency for internally situated kations to be attracted toward the poles of the cell as well as toward the chromatin; kations will therefore tend to collect in each half of the cell in a certain position of equilibrium at which these opposing tendencies are balanced; the position of these regions will vary with variations in the electrical condition of the chromatin, but will typically be somewhere between the chromatin and the extremities of the long axis of the cell. The astral centers represent these regions of highest positive potential; hence they form the centers toward which the electrical lines of force converge. Under the usual conditions they are two in number and adjoin the long axis of the cell. The positions of the two may coincide at their earliest appearance; hence an originally single aster, situated in close proximity to the nucleus, appears to divide into two which recede toward opposite poles of the cell.

The preponderance of negatively charged ions at the surface of the egg must result in a fall of surface-tension, and it is to this that cleavage on the above theory is supposedly due. That alterations of surface-tension do actually accompany a passage of the chromatin into the chromatic phase, even when normal cleavage fails to occur, seems proved by certain recent observations of E. B. Wilson[1] on artificially fertilized eggs of *Toxopneustes*. It was found that a limited number of eggs after having been exposed to the action of the fertilizing solution (mixture of equal parts sea water and 12 per cent. magnesium chloride) do not segment (as do the majority of eggs thus treated) but undergo the following abnormal changes: "the nuclear area gives rise to a single radiation or monaster which never resolves itself into a bipolar figure. Such eggs never properly segment, but pass through regularly alternating phases of nuclear transformation parallel to those of progressively dividing eggs." That is, first the nucleus enters the resting phase and the astral radiations become greatly reduced; then the nuclear membrane again disappears and the astral radiations regain their original prominence; this is again followed by the reconstruction of the resting nucleus and the reduction of the radiations. The above cycle of changes may occur several times in succession in a single egg. At each disappearance of the nuclear membrane a group of granules appears in the clear center of the aster; these are believed to be chromosomes. These bodies progressively multiply by longitudinal division until finally they may become very numerous. The important fact from our present

An interesting incidental result of these conditions is a tendency for any minute electrically negative particles casually present in the cytoplasm to be drawn along the lines of force toward the regions of highest positive potential. Here such particles must gather and remain, and in stained preparations they may present the appearance of prominent deeply staining bodies occupying the astral centers. On the above theory the centrosomes originate in some such manner as this. The affinity exhibited by these bodies for basic or nuclear dyes may be regarded as additional evidence of their acidity and electrical negativity. It should be remarked that other authors have regarded the centrosomes as formed by the aggregation of centripetally moving microsomes (cf. Bürger, *Anatomischer Anzeiger*, 1892, p. 222). On the above theory this migration is due to electrical influences. Later, however, I hope to treat these and related questions in a somewhat less summary manner.

[1] Wilson, *loc. cit.*, pp. 546, 547.

standpoint is that "*during the telophase the egg frequently becomes amœboid*, and may even make an abortive attempt to divide. In the later stages in some cases it may actually divide into a number of irregular masses, only one of which contains a nucleus (Fig. 8, *i*), but which here again completely fuse together." These phenomena are almost unquestionably the result of an alteration of surface-tension; at all events they are precisely what might be expected to occur if such alteration in surface-tension were to take place. It may therefore be regarded as an established fact of observation that during the chromatic nuclear phase certain influences are active which produce alterations in the surface-tension of the egg. That these influences are of an electrical nature may, in view of the above facts and considerations, be regarded as at least highly probable.

The increase in the surface-area of the egg during division may be held to denote a general decrease of surface-tension. But a perfectly uniform decrease of tension over the entire surface could lead to no change of form; this is evident from the fact that fluid droplets of unequal surface-tension alike tend to assume the spherical form when under the sole influence of such surface-tension, provided this is uniform at all portions of the surface. Changes of form in such droplets result when the surface-tension becomes unequal at different regions of the surface. Thus if the tension is lowered over a small circumscribed area (as appears for instance to occur in the egg during the formation of the polar bodies) the fluid tends to flow outward or be pressed outward at that region — the internal pressure being there insufficiently compensated for the retention of the spherical form — and an amœboid projection is the result. Perfectly definite changes of form, such as occur in cleavage, imply a correspondingly definite localization of the areas of lowered surface-tension. In the case of the egg various indications — such, for example, as the preliminary elongation in the direction of the spindle-axis — point to the conclusion that surface-tension is primarily lowered at the two sides of the egg opposite the astral centers. From the position of the asters during the metaphase and telophase it is to be expected on the present theory that the surface negative charge will be densest near the regions adjoining the long axis of the

egg, and that there the surface-tension will accordingly be lowered to the greatest degree. It is also clear that the influence in these regions will increase as the daughter groups of chromosomes approach the poles since the inductive action of the chromatin on the cell-surface must increase as the interval between the two diminishes. The surface-tension at the regions adjoining the astral centers must therefore decrease still further as the daughter groups of chromosomes approach the surface; in other words, the difference between the surface-tension at the polar and at the equatorial regions of the egg (speaking with reference to the spindle axis) progressively increases as the groups of chromosomes diverge. Eventually the egg is surrounded by an equatorial surface-zone possessing a distinctly higher tension — *i. e.*, a stronger tendency to contract — than the polar surface-areas. The effect is naturally the same as would be produced by the presence of a *constricting band* surrounding the equator; a "cleavage-furrow" appears which progressively deepens until complete bipartition is effected.[1]

Cleavage, on this theory, is the result of an inequality of surface-tension between polar and equatorial regions of the dividing cell, due to a greater lowering of surface-tension at the poles than at the equator. This diminution of surface-tension is dependent on the ions present in the protoplasm, hence withdrawal of these, as by the use of non-electrolyte-solutions, prevents cleavage, and, by heightening the normal surface-tension, tends to favor fusion of adjacent blastomeres.

Why similar effects should follow withdrawal of water and compression is not clear. It might be suggested that the effect of compression is an instance of the second of Lippmann's laws cited by Ostwald[2] in his "Lehrbuch": if by mechanical means the surface of the fluid (with tension lowered by the electrical double

[1] So early as 1876 Bütschli (*Abhandl. d. Senkenbergschen Naturf. Ges.*, Bd. 10) referred division to changes of surface-tension due to influences emanating from the astral centers. In his recent paper (*Archiv für Entwicklungsmechanik*, 10, 1900) the equatorial constriction is ascribed to a relative increase in the surface-tension of the equatorial zone of the egg. Conklin (*Journal of the Academy of Natural Sciences of Philadelphia*, Second Series, Vol. XII., Part I., pp. 95 et seq.) is in essential agreement with Bütschli so far as regards the immediate origin of the cleavage furrow.

[2] Ostwald, *l. c.*, p. 923; see also *ibid.*, p. 929.

layer) is increased, the electrical potential-difference of the surface also alters in such a sense as to resist a continued increase of surface, *i. e.*, surface-tension is increased. The increase of surface due to compression of the eggs — supposing the conditions to be of the above kind — must therefore heighten the normal surface-tension ; it will consequently promote fusion. With regard to the effects of withdrawal of water and raising of temperature I have no suggestions to offer at present. Withdrawal of water would result in a decrease in the number of dissociated ions, but it is doubtful if the effect could be attributed to this cause alone.

IV. Concluding Remarks.

On the view briefly sketched above mitosis is an incidental consequence of the passage of the chromatin into the strongly acid and chromatic phase ; this change involves the acquisition by the chromatin of a negative charge of considerable potential, as a result of whose inductive action there ensues a redistribution of the ions in the cytoplasm with the production of certain differences of electrical potential. To these potential-differences are due the appearance of the astral radiations and the diminution of surface-tension that leads to cleavage.

If this theory is well-founded it is evident that the ultimate determining conditions of mitosis must be sought in the conditions that control the chemical changes in the chromatin, especially those affecting the proportion of nucleic acid in this substance. In the mature egg the passage into the chromatic state normally follows the introduction of a spermatozoön ; but the same change may be artificially induced, as shown by J. Loeb and his collaborators, by withdrawal of water, action of certain electrolytes, or even by mechanical agitation. The change is of a metabolic nature and as such is presumably dependent in large part on the action of ferments. We may assume that the liberation of the enzymes concerned, or their activity when liberated, may be dependent on the presence of certain electrolytes. Further speculation on this problem, however, seems premature at present.

Only the most general conditions of mitosis have been considered above. It is evident that, among other conditions, the

state of permeability of the egg-membrane to ions, and the metabolic changes in the cytoplasm, resulting as these undoubtedly do in the production of ions, must be considered in a complete theory. It should also be borne in mind that potential-differences in the cytoplasm may conceivably originate otherwise than by induction;[1] that this is actually so is indicated by the phenomena of artificial asters or asters in enucleated eggs (cf. Morgan, Ziegler, Wilson). It seems probable however that in mitosis induction plays the chief part in the production of radiations; the formation of the sperm-aster appears to be another instance of the same phenomenon. In either case astral radiations are to be regarded as the visible expression of local differences of electrical potential within the cytoplasm.

[1] The localized production (*e. g.*, as an excretory product) of any electrolyte having ions of decidedly different migration-rates would have this effect; it would be especially marked in the case of acids (*e. g.*, lactic acid) on account of the high speed of migration of the hydrogen ion.

THE STRUCTURE AND SIGNIFICANCE OF VESTIGIAL WINGS AMONG INSECTS.

CHARLES T. BRUES.

Although considerable attention has been paid by entomologists to the structure and development of the wings of insects, but few observers have ever given any careful descriptions of insect wings which are in a vestigial condition. The well-marked constancy of the wing structure and its use as an aid to classification has long been recognized, so that one can not fail to suspect *a priori* that there should be a considerable range of variation in the structure of vestigial[1] wings. That the wings are exceedingly important organs has never been questioned, indeed, it is a generally accepted view that they are one of the most important factors in making the Insecta such a dominant group, and the causes which induce their atrophy must undoubtedly be deep-seated. Moreover, from the extent of degeneration it should be possible to obtain some knowledge as to the length of time during which such causes may have been acting. How far such a method may be relied upon, it is my present desire to show.

In a recent paper upon the reduction of wings among the Diptera Bezzi ('00) has shown that there are two categories into which we may divide such Diptera accordingly to the apparent cause of the wingless condition, viz.: First, cases brought about by external parasitism. Second, those induced by a secluded environment. It is possible to include almost all the general types of wingless insects under these two heads, except in a few cases where other influences seem to be at work.

The examination of a number of myrmecophilous Hymenoptera and Diptera has called my attention to the fact that several rather clearly defined types of vestigial wing structure may be

[1] The term vestigial is here used instead of rudimentary. The latter word has often been employed in this connection by entomologists, but with evident impropriety, as it should apply in its strict sense only to organs still in process of ontogenetic development.

recognized. Extending these observations to include other insects also, it seems possible to distribute all these cases into three categories as follows:

1. Wings having essentially a pupal character, *i. e.*, developing as normal wings up to the pupal stage but failing to expand.

2. Wings essentially normal, except for their smaller size and less complex venation; sometimes even developing a color pattern, or possessing unique and quite distinctive characters.

3. Wings consisting of little more than a hollow bag and giving no clue from their appearance as to the probable wing structure of their ancestors. (Comparable in a way to the halteres of the Diptera.)

Of these three groups, the first would seem to indicate the most recently acquired brachypterous condition, and the third apparently the one just preceding the totally apterous state. The latter should therefore be phylogenetically the oldest.

The Trend of Phylogenetic Development in Subapterous Forms.

In flying insects where the wings are of supreme importance their structure is very constant for each form; but as soon as they become vestigial to such an extent that they are no longer available for their only function, that of flight, they are a useless burden, so that once this stage has been reached natural selection should rapidly remove them entirely. This is no doubt the case, for insects with wings just too short for use in flight are very rarely met with. Of these exceptions two groups can be defined:

1. Forms where the wings have suddenly developed characters which make them of use in some other direction.[1]

2. In groups which seem to be undergoing rapid and remarkable phylogenetic changes, *e. g.*, the Phoridæ among the Diptera.

The great preponderance of the forms which have wings of the third type (vide *supra*) shows that there is a rather sharply defined point where they become so vestigial that they are no

[1] Examples of this can be seen in widely separated groups. A notable case is that of a chalcid fly, *Eupelmus rhizophelus*, considered in the sequel.

longer acted upon by natural selection. This also is seen in widely separated groups, *e. g.*, Coleoptera (*Zopherus*) and Diptera (*Ecitomyia*), where the wing is simply a slender, hollow bag of very simple structure.

The fact that in this extremely reduced state they resemble the halteres of Diptera is interesting as affording evidence from another source that the halteres are the vestiges of the second pair of wings which have acquired the new function of equilibration in the Diptera.

The Causes Inducing the Atrophy of the Wings.

Recently Dewitz ('02) has published the results of some experiments upon certain wasps (*Polistes*), where he succeeded in obtaining artificially, specimens of the *Polistes* with abortive wings. These were obtained by subjecting the young larvæ to a low temperature for a considerable period, by laying the nest upon a cake of ice. After undergoing this treatment while still young the insects developed only the stumps of wings on attaining the adult state. Whether the low temperature in this case acts in a way especially to retard the development of the wings other than by causing a general weakness of the body seems to me very doubtful. The anlagen of the wings are present in the pupa and the last and supreme act of an insect on casting off its pupal skin is properly to expand its wings. If its store of energy has been depleted by untoward conditions during its larval life they are the parts which become abnormal. Thus expansion may take place only imperfectly or not at all if the organism has not vigor enough to expand them. I have known artificial conditions of various sorts such as extreme dryness and hot, damp air in closed jars to cause specimens with deformed wings. Under natural conditions such freaks also occur, although much more rarely. During the past summer I have collected a specimen of *Ammophila urnaria* and also one of *Sphex pennsylvanica*, in which the wings were very small and much deformed. Such abnormalities are, however, fundamentally different from normally reduced wings and their structure is not constant. Moreover, such sudden variations without corresponding changes in habits can never be preserved by natural selection.

Dewitz[1] associates a diminution of the oxidation processes in the body with the wingless condition, which is borne out by the comparative anatomy of the tracheal system, but as to which is cause and which effect it is not so easy to decide. As actively flying insects require more air to aërate their blood, they naturally have more extensive tracheæ and respiratory sacs to supply it, so that it seems only proper to consider that in isolated genera or species among a winged group of insects it is the wings and not the tracheæ hidden inside the body which have first been influenced by external conditions and caused to change their form.

In the case of males of certain species which are winged while the females are wingless the short life of the male and the necessity of his seeking the female are sufficient to account for the female being the sex to lose the wings first in becoming adapted to the environment.[2]

It has long been recognized that insects inhabiting certain regions are more apt to be wingless or subapterous than their relatives living under other surroundings; notably the forms inhabiting oceanic islands, mountain tops and of deserts or arid regions tend to have the wings short or wanting. These seem to be the only anomalous cases, for all others can be traced to parasitism or secluded environment. The well-known explanation of Wallace that the great numbers of insects blown out to sea and thus destroyed has caused forms that do not fly or are wingless to be saved at the expense of the more active forms, is regarded by Dewitz ('02) as having little weight. It is true that actual observations upon this point are not numerous, but the immense numbers of dead insects that are often cast up on beaches after a severe storm show that this must be a very important factor. Needham ('00) has given interesting data upon this subject in a recent paper on the insect drift of Lake Michigan in which he describes the enormous numbers of insects cast up on certain portions of the beach of Lake Michigan.[3]

Isolated mountain peaks present similar conditions, although here the mortality can not be so great. A windstorm in such

[1] *Loc. cit.*

[2] See also note under *Dicælus*, page 187.

[3] Leconte has described similar phenomena on Lake Superior beaches.

a locality would more readily remove them from their proper environment than in the lowlands, and consequently from conditions favorable for the growth of their offspring. This would favor wingless forms.

As wingless insects are rarer in cold than in warm regions, the cold does not seem to be a possible factor.

The wingless condition of desert forms has never, I think, been satisfactorily explained, although it is the expression of a well-marked tendency.[1]

A secluded environment very often induces the atrophy of wings. This is seen most strikingly among myrmecophilous and cave insects. There can be but little doubt that in such cases wings are nearly always an inconvenience or even danger. Thus we find that all blind insects are also wingless, wings being evidently detrimental to safety when unguided by vision.

The influence of external parasitism apon the wings is so well recognized that it need only be recalled in the present connection.

From the fact previously alluded to, that the loss of wings is at first very rapid and then suddenly becomes extremely slow when they have reached a very vestigial condition, it can be readily seen that attempts to ascertain the phylogenetic age of a certain wingless type must be very difficult except in a very few cases. Added to this the great differences in plasticity among the widely separated groups with which we have to deal add further difficulties. Nevertheless, by comparing the various cases considered in the second portion of this paper, it is readily seen that the loss of wings must be very readily brought about, for nearly every apterous or subapterous form has a closely related form living under apparently nearly the same conditions, which is winged. Moreover, the loss of wings is usually accompanied by only slight changes in the external morphology of the insect.[2]

[1] In this connection it may be mentioned not as a full explanation, but as a fact which may bear upon this question; that in wingless coleoptera such as *Eleodes* and allied forms the tight-fitting and immovable elytra must prevent to a great extent the evaporation of water from the body and thus enable them better to withstand long and severe droughts. The fact that *Eleodes* also occurs in certain moist regions can be understood when we remember that arid regions seem to be the centers of distribution for such forms.

[2] In many apterous coleoptera a correlated change is the shortening of the metathoracic or wing-bearing segment.

Thus the causes must be such as affect the wings alone. It seems therefore that we may be justified in saying that it is the result of certain mechanical influences of the environment affecting the wings alone, which causes their loss. This may be accomplished either by hindering the organism in its movements [1] or by laying it open to removal from its proper environment.[2]

First Category, "Pupal" Wings.

Where the development of the wings proceeds normally up to the formation of the pupa and is then suddenly arrested, the adult wings resemble those of the pupa in general detail and form except that they are more heavily chitinized. They occur in certain flies belonging to the family Phoridæ [3] and indicate the first step toward their total atrophy. The species with this form of wings resemble more closely in other morphological details fully winged forms than do their relatives whose wings fall into the second and third categories.

The cases of wingless grasshoppers afford beautiful examples of this persistence of the wing character of the previous instar. In these Orthoptera the wings of subapterous species cannot be distinguished from those of the earlier instars of fully winged species by structure alone. Were it not for the fact that there is a reversion of the relative positions of the two pairs of wings [4] at the last ecdysis one could not decide from a mere examination of the wings whether a certain form is larval or adult.

Empyris subapterus M. et B. MSS., ♀ (Proctotrupidæ) (Fig. 2).

The wings of this species, like those of many other Proctotrupidæ, are very short, but still retain a nearly normal system of neuration. The wing membrane has been lost, while the venation remains undisturbed. This gives the wings a distinctly pupal character.

[1] That the wings are an inconvenience to life under certain conditions is evinced by the well-known fact that the fertile queens of ants actually tear off the wings on beginning their underground life.

[2] In the cases of insects inhabiting small oceanic islands, mountain tops and deserts, the possible habitat for a species is usually very limited.

[3] Notably the genera *Psyllomyia* Lw. and *Commoptera* Brues.

[4] In all instars except the last, the posterior wings lie above the anterior ones, which position is reversed in the adult.

Megaspilus sp. indescr. (Proctotrupidæ) (Fig. 3).

In a small subapterous species of *Megaspilus* is seen the very common phenomenon of a micropterous species closely resembling other fully winged forms. Many other cases of this could be cited among this group of Hymenoptera.

Apteropompilus (?) sp. indescr. (Fig. 4).

In a most remarkable nearly wingless species of Pompilidæ which occurs in Texas, the venation is more complex than that of *Empyris*, but much simpler than in other genera of Pompilidæ. In this case the wings are very small, being scarcely visible without a strong lens and the thorax is narrower than usual, due doubtless to the slight devolopment of the wing muscles. In this family no intermediate forms are known between this and the fully winged species, all of which fly with great activity. Running has taken the place of flight, a tendency which is seen in many of these sand-wasps which are fully winged.

Second Category.

The following examples have been chosen to illustrate the condition of wings which are much reduced in size, but still retain very well-defined and distinctive characters.

1. *Mutilla grandiceps* Blake, ♂ (Fig. 1).

This species of *Mutilla* is a rare exception among the males of this genus of velvet ants, for like the females of all species, it is incapable of flight. Among all the three or four hundred species of this cosmopolitan group, the rule of wingless females and perfectly winged males holds with only two or three exceptions. From the figure it can be seen that the wings are well proportioned and still retain the hooks upon the anterior margin of the hind pair. The venation is much confused and not plainly defined. The wings while very small in comparison to the size of the insects (reaching scarcely to the tip of the thorax) recall very strongly the normal wings of certain Proctotrupidæ and Chalcididæ where the venation is often completely lost.

2. *Henicopygus subapterus* Ashm. (Chalcididæ) (Fig. 6).

The wings of this insect are very small and evidently useless for flight, but nevertheless have a very distinct venation properly pro-

portioned to their size and exhibit a sharply defined color pattern of hyaline and fuscous patches. They are normal wings in miniature.

3. *Eupelmus rhizophelus* Ashm. (Chalcididæ) (Fig. 7).

One of the most remarkable modifications which I have observed occurs in this insect. The wings which are much reduced in size are suddenly bent upwards at a right angle near the middle and project like two great spines at the apex of the metathorax. The basal half is testaceous in color and flat, while the raised extremities are black and somewhat curled with acute tips. What their function may be I will not venture to suggest, but their extraordinary form leaves little doubt that they are adapted to some special use.

4. Numerous Diptera, *e. g.* Phoridæ (see Brues, '02) and the genus *Termitoxenia*, Wasmann (Fig. 10) show remarkable modifications of the wings which evidently fulfill secondary functions in these insects.

Third Category.

Although all wings falling into this group are necessarily without salient characters, they show a considerable range of variation, which is not apparently correlated, however, with their systematic relationships.

1. *Pasimachus punctulatus* Hald. (Coleoptera) (Figs. 12, 13, 14).

In this carabid beetle the wings are larger than in *Dicælus* (see below) and their larger size is accompanied by a more elaborate form and much greater range of variation, as can be seen from the accompanying figures. They have apparently not yet reached the final minimum size and simple shape. By mounting the wings of specimens which have been preserved in formalin, in glycerine jelly, the tracheation of the wing can be readily made out. A drawing made from such a preparation is shown in Fig. 14. The form of the single trachea was found to be almost identical in several wings; it is a simple tube and not dendriform except for a few slender branches at the tip and at irregular intervals near the middle of the wing where the diameter of the trachea is greatest. The tænidia are very distinct and the tube is considerably coiled upon itself, being somewhat longer than the wing. The development of the wing nervures has evidently

been stopped soon after the trachea began to push out into the wing, and the subsequent changes in the trachea not taking place, it has retained its primitive structure. The persistence of a trachea in this manner with its tænidium in an adult insect wing does not seem to have been previously recorded and is especially interesting in this case as it shows the retention of a distinctly pupal character in the imaginal instar; *i. e.*, of arrested development in the wing and not of perfect development on a smaller scale.

2. *Dicælus splendidus* (Coleoptera, Carabidæ) (Fig. 16).

In this form the wings are similar in the two sexes. They are short, slightly over two millimeters in length or one-sixth the body length, and broadly attached to the integument of the metathorax at their bases. The basal articulation is broad and not at all flexible, and as the wings themselves are very strongly chitinized, they remain immovably fixed and cover the posterolateral angles of the metathorax. The membrane forming the dorsal wall of the first abdominal segment is pushed inward to give space for the wing, which is quite thick.

Since the other species of this genus, which are winged, make but little use of their wings, it is natural to expect that here both sexes would have the wings reduced to an equal degree, for we are not dealing with a male which flies in search of the female. This points to the idea that it is the difference in the necessary activities of the two sexes that usually causes the male to retain the power of flight longer than the female, and not an inherent morphological or physiological tendency as has been suggested.

3. *Eleodes* sp. (?) (Coleoptera) (Fig. 17).

One of the common Texan species of this large Tenebrionid genus which was examined has extremely short, often bilobed wings which appear simply as slightly projecting protuberances of the metathoracic wall. *Eleodes tricostata* Say and other species have very similar wings.

4. *Zopherus* sp. (?) (Fig. 11).

In this strange Tenebrionid we do not find, as might be expected from the complete coalescence of the elytra, a complete absence of wings. There are structures several millimeters in length, forming thin and delicate flattened sacs which usually

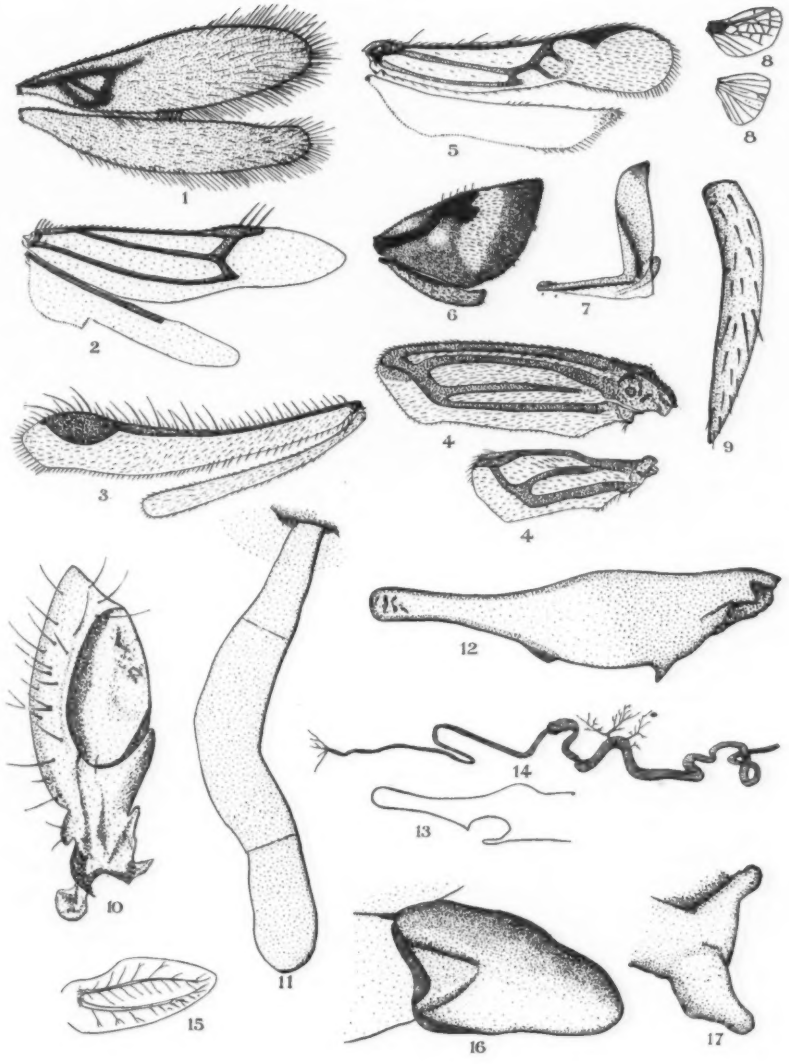
show two sharp transverse creases, and very different from the stumps of thick chitin seen in *Eleodes*.

4. *Ecitomyia Wheeleri* Brues (Fig. 9).

The simple type of a hollow bag is seen here in its plainest form, the wing is slightly curled so as to be convex dorsally and concave below, while the upper side is covered with scattered bristles. Otherwise it is structureless. Another Dipteron, *Eretmoptera* recently described by Kellogg has small wings very similar to those of *Ecitomyia*.

EXPLANATION OF PLATE.

FIG. 1. Wing of *Mutilla grandiceps* Bl. ♂.
FIG. 2. Wing of *Empyris subapterus* (M. et B. MSS.), ♀.
FIG. 3. Wing of *Megaspilus* sp. indescr.
FIG. 4. Wing of *Apteropompilus* (?) sp. indescr.
FIG. 5. Wing of a subapterous Braconid. (Chelonus)
FIG. 6. Wing of *Henicopygus subapterus*, ♀, Ashm.
FIG. 7. Wing of *Eupelmus rhizophelus*, ♀, Ashm.
FIG. 8. Wing of a micropterous ♂ of *Isogenus nubecula*. (After Sharp.)
FIG. 9. Wing of *Ecitomyia Wheeleri* Brues, ♀.
FIG. 10. Wing of *Termitoxenia Heimi* Wasm. (♀ ?). (After Wasmann.)
FIG. 11. Wing of *Zopherus* sp.?
FIG. 12. Wing of *Pasimachus punctulatus*.
FIG. 13. Wing of *Pasimachus punctulatus*, less enlarged.
FIG. 14. Tracheation of apical part of wing shown in Fig. 12.
FIG. 15. Wing of *Pœcilium affinis*, ♂. (After Sharp.)
FIG. 16. Wing of *Dicælus splendidus*.
FIG. 17. Wing of *Eleodes* sp. (?).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NEW YORK, December 19, 1902.

DEATH-FEIGNING IN TERRESTRIAL AMPHIPODS.[1]

S. J. HOLMES.

The instinct of feigning death is extensively distributed throughout the animal kingdom and appears to have arisen sporadically in several groups of animals. It crops out here and there among unrelated forms in such a manner that it is evident that the instinct has arisen independently along many different lines of descent. Various theories of the origin of this instinct have been advanced, but it is by no means evident that the method of its development has been in all cases the same. While at Wood's Hole, Mass., during the past summer my attention was drawn to the death-feigning of the large terrestrial amphipods which occur there in great numbers on the beach, and I was led to study the behavior of these animals with the end of ascertaining, if possible, how their peculiar instinct may have arisen. The family Orchestiidæ, to which the terrestial Amphipoda belong, is partly terrestrial and partly aquatic, and the terrestial forms are, as a rule, confined to within a short distance of the seashore where they live in an atmosphere heavily charged with moisture. The instincts of the terrestrial Orchestiidæ which adapt them to their peculiar habitat must have arisen through certain modifications of the behavior of their aquatic relatives; and as there are several terrestrial and two aquatic species of this family found at Wood's Hole the attempt was made to gain light upon the problem by a comparative study of the behavior of these different forms. I was unable to study the behavior of one of the large sand-fleas, *Talorchestia megalophthalma*, as I failed to obtain any specimens in a living condition. The allied species *T. longicornis* is much more abundant at Wood's Hole and may easily be obtained in any desired quantity. It is the only species observed in which the death-feigning instinct is clearly shown, but the other species, as will appear below, manifest the same fundamental peculiarity, though in an altered form.

[1] From the Zoölogical Department of the University of Michigan, Ann Arbor, Mich.

Talorchestia longicornis during the day lies curled up in burrows in the sand situated usually a short distance above high-tide mark where the sand is loose and dry on the surface but moist an inch or more below. The animal remains all day in an apparent stupor much like the sleep of higher animals. The feeding time is at night when *Talorchestias* come out of their holes in swarms and run or hop in a lively manner over the sea-weed near the water's edge. When dug out of its burrows in the daytime *Talorchestia* may lie curled up in its original position, apparently unawakened by the disturbance. Frequently it will make a few hops in the sand and then curl up and lie perfectly quiet. In assuming this condition the body is not only strongly flexed but the legs are drawn up and the antennæ are bent under the thorax, the whole animal assuming as compact a form as possible. While in this attitude *Talorchestia* may be rolled about, picked up and handled, often with considerable roughness, without betraying any signs of animation. It feigns death as perfectly as many of the insects or spiders. After lying in the death-feigning attitude for some time the animal quickly jumps up and scampers away. Waking up, however, is not instantaneous, but is preceded by certain symptoms which may readily be observed if the creature is closely watched. There is first a nervous twitch here and there, then a slight unbending of the antennæ and a straightening of the legs and body—all of which indicate a slight relaxation of the muscular tension under which the animal labors—and finally a sudden spring and attempt to escape. The animal may be induced to feign death by holding it quiet for a moment in the hand or by placing it in the sand. It will feign death many times in immediate succession but the duration of the response becomes on the average less the more often it is caused to repeat the performance. After *Talorchestia* feigns death several times it is more difficult to bring about the response. As it becomes exhausted the death-feigning attitudes become less typical and the body more relaxed and flaccid. The animal is by no means passive while exhibiting this instinct but is in a state of muscular tension; and this condition is, I believe, very common in the death-feigning of other animals. The contact of solid bodies apparently favors the continuation of the

feint; at least the duration of the feint is increased when the body of the animal is surrounded with sand or small stones. This fact was determined by placing *Talorchestias* which had feigned death upon a flat surface and counting the number of seconds the feint continued and then comparing the series of observations thus obtained with an equal number of observations made upon specimens partly covered with sand or small stones. Fifty trials were made and the average duration of the feint of the specimens partly surrounded by sand or small stones was found to be much longer than that of the specimens lying on a flat surface. As *Talorchestia* is coming out of its feint a slight pressure or contact causes it to resume feigning.

There is little evidence that the death-feigning of *Talorchestia* is in any way connected with a conscious attempt at deception. Such a performance is utterly beyond what the degree of psychic development which the Amphipoda have probably attained would lead us to expect. The instinctive action of *Talorchestia* which seems most like an intelligent attempt to deceive an enemy is that of crouching upon the approach of a threatening object. *Talorchestia* when running away often crouches to the ground and lies perfectly quiet if a large object draws suddenly near. When things in its environment become quiet again the animal moves on. *Talorchestia* does not feign death upon receiving purely visual impressions; it requires contact of some sort to elicit this form of response. The same fact seems to be quite general in the death-feigning of animals, especially below the vertebrates, and it is a circumstance, I believe, of considerable significance in relation to our views of the genesis of this instinct.

The value of the death-feigning instinct in *Talorchestia* is obvious. When the animal is dug out of the sand its large size would render it an easy prey to an active bird or mammal if it attempted to seek safety in flight; by lying quiet it is, as every one knows who has dug these creatures out, very easily overlooked on account of the resemblance of its color to that of the sand around it. Its death-feigning and its protective coloration both make for concealment and consequently are of service in the life-history of the animal.

The smaller sand-fleas *Orchestia palustris* and *O. agilis*, live

in a different situation on the beach from that occupied by the the species of *Talorchestia*. The little *O. agilis* is found in countless numbers beneath the piles of seaweed near the water's edge. This species, as its name implies, is exceedingly active. When disturbed it jumps very rapidly and to such lengths that capturing the creature is an exceedingly difficult undertaking. An enemy which could easily catch the large *Talorchestias* would find the attempt to capture *O. agilis* an unprofitable pursuit. *O. agilis* generally continues hopping until it alights in a place where it can readily get under some object or wedge itself between bodies, so that it secures contact on a considerable surface of its body. Contact seems to exercise a peculiar influence upon this organism, a sort of hypnotic effect apparently, which induces it to flex its body, bend the antennæ downward and lie quiet. The body and antennæ are not so strongly flexed as in the death-feigning of *Talorchestia*, but the same actions are performed though not carried so far. When lying thus *O. agilis* may be disturbed slightly without performing any movement, but an attempt to pick it up or push it about will cause it to quickly "come to" and hop away in the most lively manner. This instinct of *O. agilis* to get into close contact with solid objects is an expression of the strong thigmotactic tendency found among amphipods in general. It is a tendency especially marked in the aquatic representative of the Orchestiidæ, *Allorchestes littoralis*. This species is commonly found under or among stones quite high up on the beach above the range of any of the other aquatic species, thereby showing an approach toward a terrestrial habit. When taken out of the water it is able to perform the exceptional feat of walking upright without falling upon its side, although this is accomplished with some difficulty, and of making leaps into the air like its terrestial relatives. When disturbed it usually moves away by gliding on its side, a movement very common among amphipods which is performed by alternately flexing and extending the abdomen. The efforts are continued until they bring the creature into some niche or crevice where the contact sought for is obtained; then it curls up and lies quiet. The thigmotactic reactions of amphipods keep these animals among the seaweeds and rocks where they secure protection and obtain

food. The behavior of the terrestrial *O. agilis* in relation to solid objects is little modified beyond that of the aquatic species. The thigmotaxis of this form is certainly protective in function, not only by enabling the animal to escape detection by lying quiet, but by leading it into situations such as under stones or into crevices which are inaccessible to its enemies.

The behavior of *Orchestia palustris* shows an interesting connection between that of *Talorchestia* and *O. agilis*. *O. palustris* is considerably larger than *agilis* and is not so active in its movements. It is often found in marshes some distance from the seashore. It usually endeavors to escape by running away and resorts to hopping only under necessity. The tendency to get under or between objects is as strongly developed in this species as in *agilis*, and contact has apparently a stronger quieting effect upon it. When lying quiet *O. palustris* may be poked about more or less without being aroused from its thigmotactic lethargy. Only rarely, however, can it be picked up without its making efforts to escape, although it is much less responsive than *O. agilis*. The conduct of this species is intermediate between the thigmotactic response of *agilis* and the death-feigning of *Talorchestia*. Some specimens might almost be said to possess a death-feigning instinct. The curling up of the body and the bending of the antennæ are not carried so far as in *Talorchestia*, but the same actions are performed which, if carried out in a more decided manner and persisted in longer, would result in what would commonly be called feigning death. The death-feigning instinct of *Talorchestia* is an instinct which, I believe, has its root in the thigmotactic responses common among other amphipods. One may easily conceive that by the selection generation after generation of those individuals of *O. agilis* in which the thigmotaxis is most persistent and in which the body is drawn up in the most compact form during the response a mode of behavior like the death-feigning instinct of *Talorchestia* might readily be produced.

It seems not improbable that an instinct having its phyletic root in a simple thigmotactic response may in course of time come to be comparatively independent of contact stimuli. The persistence of death-feigning in *Talorchestia* depends far less upon contact than the thigmotactic reactions of the aquatic Am-

phipoda, although, as has been pointed out above, contact still increases the duration of the feint. Contact, finally, may come to be necessary only to set up the instinctive response, having become entirely superfluous for its continuation. And if we conceive the necessary stimulus to be reduced to a single tap or even a jar we can understand how death-feigning reactions such as are found in certain beetles where the response often follows upon the slightest disturbance may have been evolved. Whether they have been so evolved is a question which it would be rash with the evidence in hand to attempt to answer. The singular circumstance that the death-feigning reaction is almost always evoked in response to some form of contact stimulus might be urged in support of such view. The instinct of feigning death has been evolved independently so many times that it is quite possible, if not probable, that it has risen by different methods in different groups of animals. The problem can be solved only by a careful comparative study of death-feigning in several related forms among the various groups of the animal kingdom in which the instinct occurs.

NOTES ON THE REVERSAL OF ASYMMETRY IN THE REGENERATION OF THE CHELÆ IN ALPHEUS HETEROCHELIS.

EDMUND B. WILSON.

We owe to Przibram[1] the interesting discovery that when the larger chela in the genus *Alpheus* (*A. dentipes*, *A. platyrrhynchus*, *A. ruber*) is removed, the chelæ undergo a reversal during the ensuing regeneration, a small chela being regenerated from the stump of the large one, while the former small chela, *which has not been injured*, is directly transformed at the first or second moult into a large one that shows the characteristic structural features of this appendage. A precisely analogous result was obtained in the annelids by Zeleny,[2] who found that after amputation of the functional operculum in *Hydroides* a rudimentary operculum was regenerated in its place, while the rudimentary operculum previously present on the opposite (uninjured) side developed directly into a functional one.

These cases are highly interesting since the reversal of asymmetry involves not merely the enlargement of a smaller structure on the uninjured side, but also profound structural and functional changes due to an injury to another part of the body.

During the summer of 1902 I had an opportunity at Beaufort, N. C.,[3] to repeat Przibram's experiments on *Alpheus heterochelis*, a form in which the differentiation between the two chelæ is extremely marked, and to make some observations on the control of the regenerative process by the nervous system. The anatomy, habits and development of this form have been carefully described by Brooks and Herrick,[4] whose observations give data having an important bearing on the facts to be described.

[1] "Experimentelle Studien über Regeneration," *Arch. für Entwkm.*, XI., 1901.

[2] "A Case of Compensatory Regeneration in the Regeneration of *Hydroides dianthus*," *Arch. für Entwkm.*, XIII., 4, 1902.

[3] I am indebted to the Hon. G. M. Bowers, United States Commissioner of Fisheries, for the privilege of occupying a table at the Beaufort Laboratory, and to Dr. Caswell Grave, director of the laboratory, for his kind coöperation.

[4] "The Embryology and Metamorphosis of the Macroura," *Mem. Nat. Acad. Sci.*, V., 4.

As shown in Fig. 1, *A*, *B*, the chelæ in this species differ very widely both in size and structure; right-handed and left-handed individuals occur in approximately equal numbers. The large or hammer chela (*B*), which is nearly or quite half the length of the whole animal exclusive of the antenna, is greatly swollen, with a deep transverse groove on either side of the propodus, and a characteristic color-pattern. Both claws are extremely stout, and show very remarkable and characteristic features. On the concave side of the dactylus is a great swollen knob, forming the "hammer" (*h*), which fits into a corresponding deep socket on the outer side of the propodus claw, overhung on the upper side by a prominent setose ridge (*p. s.*). By fully extending the dactylus and then suddenly snapping the claws together the hammer is forced into the socket with such force as to produce a surprisingly loud report, whence the popular name "pistol crab" applied to the animal in some localities (*cf.* Brooks and Herrick).

The large chela has essentially the same structure in both sexes, but the small one shows characteristic sexual differences. In the female (Fig. 2, *D*) it is very straight and slender, and relatively smaller than in the male. In the latter (Fig. 2, *A*) it is not only relatively somewhat larger, but stouter, with relatively shorter claws, the dactylus more strongly curved, and traces of the transverse grooves of the large chela are often present (Fig. 1, *A*). Its most characteristic feature in the male is the presence on both sides of the dactylus of a very marked curved ridge, bearing a series of stiff, short setæ (*d. s.*). A somewhat similar but straighter setose ridge (*p. s.*) is also present on each side of the propodus claw in a position corresponding to that of the setose ridge in the large claw. No trace of the hammer is present in either sex. Ordinarily the small chela alone is used in taking food, the large one being in the main a weapon of offense and defense, as Brooks has graphically described.

As far as the reversal of asymmetry is concerned my observations on *A. heterochelis* entirely confirm those of Przibram on the three species studied by him, but give a slightly different result in cases where both chelæ are amputated. Przibram found (*op. cit.*, p. 331) that in such cases each stump regenerates an appendage of the same type as that which has been removed (*i. e.*, no

reversal occurs) but the two are of nearly equal size. The species I have studied agrees in showing no reversal after this operation, but the hammer claw is from the first in most cases very distinctly larger than the other; though as was to be ex-

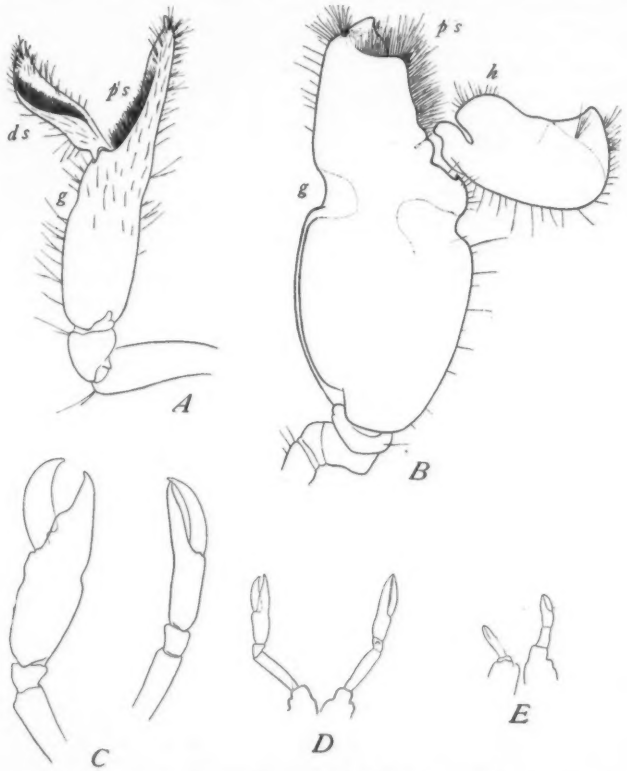

FIG. 1. Normal and regenerating chelæ of *Alpheus heterochelis*, × 4.

A, *B*. Left and right chelæ of male; *d. s.*, setose ridge of dactylus, *p. s.*, setose ridge of propodus; *h*, hammer; *g*, transverse groove, only faintly indicated in the small chela.

C. Chelæ regenerating without reversal, after removal of both appendages from a left-handed specimen; immediately after first moult, twelve days after operation.

D. Chelæ of originally left-handed individual, regenerating very slowly without reversal after removal of both chelæ; eleven days after operation.

E. Regenerating chelæ of originally left-handed individual, three days after removal of both chelæ. Differences of the chelæ already apparent; no reversal (the appendages are viewed from the lower side and hence appear reversed as compared with the other figures).

pected the unequality is very much less marked than in a normal specimen (Fig. 1, *C*, *D*, *E*). A comparison of Przibram's Figs. 20–23 (*A. dentipes*), 32–35 (*A. platyrrhynchus*) with mine shows that in point of fact the difference is one of degree only; for in both those species also the figures represent the hammer-claw as slightly larger. The most important addition I am able to make to Przibram's result is the fact that if, after removal of the large chelæ, the nerve of the remaining small chela be cut at the base, *the reversal in some cases at least does not take place, or is incomplete.*

In all, more than a hundred operations were performed, but owing to the large mortality to be expected under the conditions given by the lack of running water in a warm climate not more than half gave definite results, and I shall here report only on such cases.[1]

A first series, including a simple repetition of Przibram's work, gave the following result:

First Series.

A. Of 17 cases in which the large chela was removed, all without exception regenerated in the reversed condition, the remaining small chela being transformed into one of the large type.

B. Of 15 cases in which both chelæ were removed 14 regenerated without reversal — *i. e.*, large and small chelæ reappeared on the same respective sides as before the operation — while a single specimen regenerated reversed.

In three of the cases under *A* the larger chela (originally the smaller) formed on the uninjured side was removed soon after the moult, with the result that a second reversal took place, restoring the original condition. One of these cases is illustrated by Fig. 3, *A–C*. The animal, a female, had originally the

[1] The animals were kept singly in bowls, the water being changed once or twice daily, and were abundantly fed on oysters, which were greedily devoured. A complete permanent record of the experiments was kept by preserving every cast skin and the animal that emerged from it, so that there can be no doubt as to the condition of the animal before and after the moult. As a rule the animals were fixed soon after the first moult; but in cases where it occurred soon after the operation they were kept until the second moult. The moults occurred at intervals of 9 to 14 days.

large chela on the left side. The first moult occurred nine days after removal of the large chela; Fig. 3, *A*, shows the cast skin of the original small (right) chela, *B*, the same appendage removed three days after the moult, showing the characteristic features of the large chela (transverse groove, shortened and thickened chela, and the "hammer"). The second moult occurred six days later (nine days after the first moult); Fig. 3, *C*, shows the result, the appendage having returned to its original condition, except in size, while a chela of the large type had appeared on the other side.

In all cases the hammer-chela on its emergence from the skin of the small chela is less modified than that of a normal animal, always showing characters intermediate between those of the fully developed hammer-chela and the small chela; and the same is true of the regenerating hammer-chela after both chelæ have been removed. In general the longer the period between the operation and the ensuing moult the greater the modification of the hammer-chela. As may be seen from the figures the reformed or regenerating hammer-chela is less robust than the fully developed one, the claws are longer and less curved, the transverse groove less pronounced and the setose ridge of the propodus (*p. s.*) further removed from the tip of the claw. All these characters become more and more accentuated as the period between operation and moult increases. The shortest observed period in which the regenerating chelæ (after removal of both) showed distinct differences was three days (Fig. 1, *E*); but the difference is here still one of size and general development only. After removal of the large chela only the structural differences may become clearly apparent after five days, as shown in Fig. 2, *G*, *H* (though in this case the remodelled small chela fixed nine days after the moult has probably undergone some further change). In this interesting case the hammer-claw very clearly shows a combination of characteristic characters of the small and large chelæ. It still retains on the whole the general proportions of the small chela of the male, but is more robust, the claws are somewhat shorter, the transverse groove has appeared, and the "hammer" has begun to form. The most interesting point is the retention of the characteristic setose ridge (*d. s.*) of

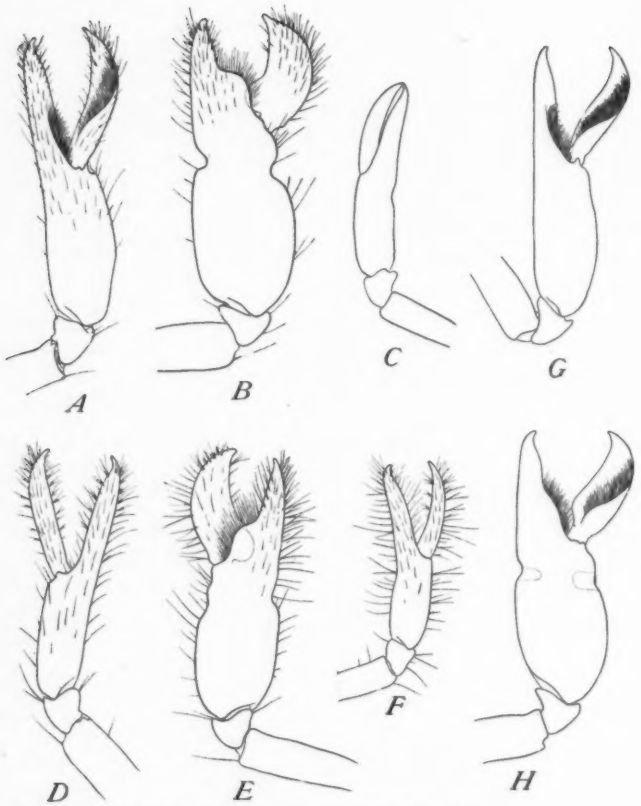

FIG. 2. Typical reversal, after removal of large chela, ×4.

A, *B*, *C*. Reversal in a male, originally left-handed; result of first moult, eleven days after removal of large chela. *A*. Cast skin of original small right chela. *B*. Right chela immediately after moult, showing transformation into hammer-chela well advanced. *C*. Left chela, immediately after moult, regenerated on the stump of the large chela removed.

D, *E*, *F*. Reversal in a female slightly larger than the specimen shown in *A–C*, originally right-handed; result of first moult, sixteen days after removal of large chela. *D*. Cast skin of original small left chela. *E*. Left chela immediately after moult, transformation less advanced than in the male. *F*. Right chela, immediately after moult, regenerated on the stump of the large chela removed.

G, *H*. Initial changes in the typical reversal of an originally left-handed male. *G*. Cast skin of right chela, after first moult, five days after removal of the large left chela. *H*. The same appendage nine days after the moult, showing remodelling in progress, but still retaining the setose ridge on the dactylus.

the dactylus of the male small chela, which is entirely absent in the more modified forms (*e. g.*, in Fig. 2, *B*, *E*). A comparison of the figures will show that in every respect the specimen is intermediate between the unmodified male small chela (Fig. 2, *A*) and the more modified form (Fig. 2, *B*). In specimens of 9 days the setose ridge of the dactylus has wholly disappeared (Fig. 3, *B*) and, except for its somewhat more slender form, is as characteristic as in those of two weeks or more (Fig. 2, *E*).

Second Series.

In a second series the same operations were performed, but in addition the nerve supplying one or both stumps was cut below the base of the appendage. In one set (*C*) the large chela was removed and the nerve of the remaining small chela on the opposite side was cut; in a second set both chelæ were removed and the nerves of both stumps cut. This operation, easily performed by means of a slender sharp-pointed scalpel, results in the first case in a complete paralysis of the remaining appendage. If after a few days control of the appendage was regained, as often happened before the ensuing moult, the operation was as a rule repeated. The mortality in operations of this type is large, and many of the specimens sooner or later cast the remaining appendage; hence only eight successful cases were obtained, and of these only two are beyond question.

C. Of eleven cases in which the large chela was removed and the nerve of the remaining small chela was cut, nine regenerated without reversal, one with reversal, and one with both chelæ of the large type.

D. Of three cases in which both chelæ were removed and the nerves of both stumps cut, all regenerated without reversal. These cases differed from those recorded under *B* only in showing a slight retardation in regeneration.

Of the first nine cases recorded under *C* seven had thrown off the small claw at a varying period before the moult and hence are without value; for such specimens have lost both chelæ. Experiments to test this point show in fact that if the large chela be removed and the smaller one be subsequently removed, after a period sufficiently long to admit of complete reversal under

ordinary conditions, the animal regenerates without reversal. For example, from a male left-handed individual the large chela was removed and twelve days later (a period long enough to effect a complete reversal, as shown by other experiments) the remaining small chela was also removed. The animal moulted nine days later (twenty-one days after the first operation) and is *not reversed* showing a typical small chela on the right side and a large one on the left, which however is less modified than usual.

The absence of reversal in the seven cases in which the remaining chela was cast may have been due to the same cause as in the above experiment; but two cases remain, that seem beyond question, though one of these did not moult quite normally and the other not at all. The first case (Fig. 3, *D–F*), a right-handed male, moulted nine days after the operation, but did not succeed in extricating the small chela from the cast skin. The appendage was, however, easily drawn out by hand, in an apparently quite healthy condition, and fixed in formalin. From the right stump of the large chela had been regenerated one of the same type (Fig. 3, *F*), which though still small, shows clearly enough all the characteristic features of the appendage. The appendage drawn out of the cast (Fig. 3, *E*) is on the whole of the small type but has lost the characteristic setose ridge of the dactylus, and shows an indication of the hammer. It is, however, far less modified than the uninjured appendage after the same period of time (Fig. 3, *B*).

The second case, which did not moult, is shown in Fig. 3, *G*, *H*, fourteen days after the operation. The appendage developed on the stump of the large chela, though small, is plainly of the large type. The small (left) chela, owing to the absence of a moult, shows no change.

The foregoing data are too meager to have a very high value, yet they render it probable that the moulding of the small chela into one of the large type, and the production of the small one on the opposite side is controlled by the nervous system — a result in accord with Herbst's remarkable observations on the regeneration of the eye in *Palæmon* and Morgan's on the regeneration of the head in *Allolobophora*. It is possible that the failure of reversal in such operations in *Alpheus* is due to a circu-

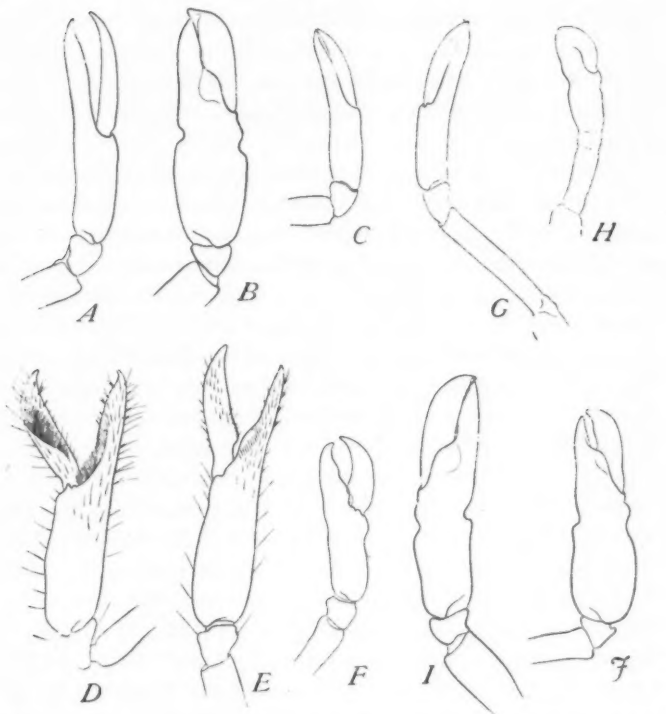

FIG. 3. Normal reversal, and partial inhibition of the reversal after section of nerve of small chela, ×4.

A, *B*, *C*. Double reversal after two successive amputations of large chela. *A*. Cast skin of right chela, female, first moult, nine days after removal of left large chela. *B*. The same appendage amputated three days after moult. *C*. Appendage regenerated on the same stump, immediately after the second moult, six days later; restoration of the original condition. The left chela is again of the large type.

D, *E*, *F*. Partial inhibition of reversal by section of the nerve of the small chela after removal of the large one; originally right-handed male. *D*. Cast skin of small chela at moult, nine days after operation. *E*. The same appendage withdrawn from the cast at the moult. *F*. The right chela, showing distinct features of the large type.

G, *H*. Partial inhibition of reversal after section of nerve. Originally a right-handed female. *G*. The small chela, fourteen days after operation; no moult. *H*. Chela of large type regenerating on stump of large chela removed.

I, *J*. Formation of chelæ transitional to large type on both sides after amputation of large (right) chela and section of nerve of remaining small chela. Originally a right-handed female. Appendages as they appeared after the first moult, fourteen days after operation. Two days before the moult the left chela showed a regain of nervous control.

latory or other non-nervous disturbance. Since, however, the appendage remains in a healthy state and emerges from the moult in a quite normal condition and without sign of atrophy, it seems more probable that the effect is directly due to the lack of nervous control.

The two exceptions recorded above are of considerable interest. In the first case the animal, originally a right-handed female, shows a perfectly typical reversed condition. In the second, the animal, originally a right-handed female, has regenerated both chelæ of the large type, showing the characteristic hammer and other distinctive characters (Fig. 3, *I*, *J*). The left chela (originally the small one) is, however, distinctly less modified than the right, being more slender, with longer and more slender claws, and is in this and other respects clearly intermediate in character between chelæ of the large and small type (Fig. 3, *I*). *Both these specimens, and these alone of those recorded, had regained partial control of the remaining appendage before the moult.* Unfortunately the record does not show precisely how long the control had been regained before the moult. The facts indicate, however, that in the first case the nervous connections had been reëstablished relatively early, so that the typical metamorphosis took place. This case probably gives an interpretation of the second one, which may be explained by supposing that in the early period, before the nervous connections were established, the stump of the large chela (right) had already partially regenerated a chela of the same type (as in the typical case); but that the transformation did not proceed so far as wholly to check the metamorphosis of the small chela (left) upon the reëstablishment of control. Both claws, therefore, reappeared of the large type, that on the right, however, being more modified than the other.

Reversal in Relation to the Secondary Sexual Characters.

The experiments bring out some interesting points in relation to the secondary sexual characters (which were not studied by Przibram) and offered data of some importance for the general interpretation of the facts. These concern especially the

structure of the small chela, which differs widely in the two sexes.[1]

In the female *Alpheus* this appendage is distinctly less modified than in the male (Fig. 2, *D*), being of slender form, with the claws straighter, weaker and longer. In the male (Fig. 2, *A*), the chela is of more robust form, the dactylus is more curved, and bears on each side a prominent curved ridge armed with a close series of strong setæ, while the fixed claw of the propodite has on each side a similar but straighter and weaker ridge also covered with setæ. Both these ridges are absent in the female. A comparison of Figs. 1, *A*, *B*, 2, *A*, *D*, clearly shows that in some of these respects the small chela of the male is intermediate in type between that of the female and the large chela, which is essentially of the same type in both sexes. This appears in the robuster form of the male chela, the occasional presence of a slight transverse groove on the propodus corresponding to the deep groove on the large chela, the greater thickness and curvature of the dactylus, and the much greater prominence of the seta-bearing ridge on the claw of the propodus, which obviously corresponds to the prominent setose ridge in the corresponding position on the large chela. The female small chela is obviously of more generalized type; and as far as can be judged from the figures of Brooks and Herrick is closely similar to both chelæ of the larval form, on their first appearance.

With these points in mind it is interesting to compare the chelæ of an animal after the first moult subsequent to removal of the large chela. *In both sexes the small chela, regenerated from the stump of the large one, is of the female type* (cf. Figs. 2, *D*, *C*, *F*) *while in both the re-forming large chela is intermediate in form between the fully developed* large chela and the small chela of the male. Furthermore, a comparison of the large chela, in regenerated individuals, shows in general that the longer the period after the operation, the greater its divergence from the small chela. Consideration of the sexual differences brings out the further interesting fact that the transformed small chela of the male for the same period of time is more highly modified than that of the female. This is clearly shown by a comparison of

[1] *Cf.* Coutière, "Les Alpheidæ," *Ann. Sci. Nat. Zoöl.*, VIII., 9, 1899.

Figs. 2, *B*, 2, *E*. The former shows the transformed small chela of the male, the latter that of the female. The former is in every respect — general proportions, depth of transverse groove, shape and proportion of the claws — more highly modified in the direction of the adult hammer-chela, though the period since the operation was eleven days, while in case of the female it was sixteen.

These facts seem to leave no doubt that the female small chela represents a relatively undeveloped or larval type, that of the male a further development of the same type, accompanied by the appearance of certain specialized secondary sexual features (setose ridge), while the large chela is the extreme of the same line of development. The male small chela is more rapidly transformed into one of the large type, common to both sexes, because it has already advanced further on this line than that of the female.

Comment.

In the above facts we probably find a basis for an interpretation of the reversal of asymmetry during regeneration. The great size of the large chela in *Alpheus*, and its importance as a weapon of offense and defense which Brooks and Herrick pointed out, suggest at once the teleological interpretation that the reversal is a device to secure the least possible delay in the restoration of an important organ by utilizing a structure already present as the foundation of the large chela. That this result is actually effected by the reversal is beyond question ; but we need not for this reason assume that the reversal has been specially acquired for this purpose. Structurally the small chela of the female represents a large chela in a state of arrested development, with hardly noticeable modifications of the larval type.[1] That of the male represents a slightly more advanced development along the same line, together with certain special modifications — notably the setose ridge upon the dactylus. In both sexes, accordingly, its transformation into a hammer-chela repre-

[1] The figures of Brooks and Herrick (*op. cit.*, Plate XX., Figs. 2, 3) of the larval form of this species are not sufficiently detailed to prove this completely, but show both chelæ of equal size and, as far as can be judged, nearly similar in form to the adult female small chela, or the regenerating small chela of both sexes.

sents, in its main features, the completion of a process that is inhibited or held in abeyance in the normal condition; though in the male this forward movement is accompanied by a regressive process which causes the disappearanee of its specific modifications (setose ridge of the dactylus). To vary the statement, the development of *Alpheus*, at first symmetrical, tends towards a state of equilibrium, characteristic of the species, which is attained through a great inequality in the size of the two chelæ and a series of structural modifications affecting especially the larger one. With the removal of the larger chela the normal equilibrium is reversed and the restorative process proceeds on both sides along the same general lines as in the normal development until a condition of normal equilibrium is restored. A similar interpretation will perhaps apply to Brachyura examined in Przibram's second interesting communication,[1] where it is shown that after the removal of either or both chelæ the regenerated form is always that of the less modified form (which is usually also the smaller). Where only the crushing chela is removed the remaining chela is not transformed into one of the other type; hence no reversal occurs. This case is clearly intermediate between that of *Alpheus*, in which complete reversal occurs, and *Homarus*, in which, as Przibram shows, no reversal occurs, each chela regenerating one of its own type whether one or both is removed. The Brachyura in question (*Carcinus*, *Portunus*, *Eriphia*) exhibit one element of the reversal, namely, the formation of a less modified chela from the stump of the crushing-chela; but fail to complete the metamorphosis of the remaining one. This may be due either to the slowness of the process (which may require a longer time for its completion than that during which the animals were kept under observation) or to a greater rigidity of organization. In *Homarus* the regenerating crushing claw is not in its very early stages recognizable as such, but is of the embryonic type; it assumes many of its characteristic features very early, but for a long period remains of a type intermediate between the two forms of chelæ (*cf.* Przibram's Figs. 13, 14). No reversal occurs. These various cases

[1] "Experimentelle Studien über Regeneration," II.; *Arch. Entwkm.*, XIII., 1901–02.

obviously form a series, at one extreme of which the large chela, after its removal, reappears and remains permanently of the small type, with transformation of the small chela into one of the large type (*Alpheus*); in the Brachyura the first process occurs but not (as far as the observations show) the second; while in *Homarus* the large chela shows transitional characters from a very early period.

In the case of *Alpheus* it is a tempting conclusion that the initial factor (*Auslösung*) that sets in motion the complex process of differentiation of which either side is capable, is primarily only a difference in the amount of material on the two sides.

Mr. C. T. Brues has at my suggestion undertaken a study of the internal changes and has determined the interesting and unexpected fact that the nerves supplying the two chelæ do not differ perceptibly in size; and they appear further not to differ in the number of the component nerve-fibers or the size of the ganglionic centers from which they proceed. As far as the nervous system is concerned, therefore, the adult appears to retain the bilateral symmetry of the larval form, the asymmetry arising through hypertrophy in other tissues, Removal of the large chela obviously reverses the asymmetry in respect to these tissues, and must temporarily at least, lead to a functional nervous difference on the two sides which may be accountable for the release of development in the small chela. This is, however, only a suggestion that must await further test.

The interest of the general interpretation offered above, which is essentially similar to the one suggested by Zeleny in the case of *Hydroides*, seems to me to lie in the explanation that it offers, of a regulative process of undoubtedly high utility to the animal, that is in the main effected by the same factors as those operative in the normal development; and it seems not unlikely that many regulative processes in regeneration may be capable of a like interpretation.

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A PRELIMINARY NOTE ON THE POSITION OF THE PRIMITIVE STREAK, AND ITS RELATION TO THE EMBRYO OF THE CHICK.

FLORENCE PEEBLES.

At the end of the eighth to tenth hour of incubation of the hen's egg, the primitive streak begins to appear as an opaque line extending from the inner edge of the area pellucida to the center of the blastoderm. Between the tenth and eighteenth hours the primitive streak extends in length, until it covers a distance equal to two-thirds of the entire length of the area pellucida. While this increase in length is taking place the primitive groove forms, and at about the twentieth hour the head process is visible as a thickened line extending anteriorly from the primitive streak. I have endeavored to determine, experimentally, the direction of growth, and the exact position of the primitive streak in relation to the later embryo.

Assheton concluded from the results of his experiments that the central point of the area pellucida, before it becomes pear-shaped, represents the anterior end of the primitive streak, and that the latter develops "from the portion of the unincubated blastoderm which lies between the center of the blastoderm and the posterior margin of the area pellucida." Assheton does not mention the number of hours that the eggs were incubated, so that we do not know whether this central point represents the anterior end of the young primitive streak, or the point from which, later, the head process extends. His figures indicate, however, that the central point of the area pellucida represents the anterior end of the completely formed primitive streak of the eighteenth hour, before the head process appears.

In another series of experiments Assheton allowed the embryos to remain in the incubator twenty-four to forty hours after a sable hair had been inserted in the center of the unincubated blastoderm. In the embryos of twenty-four hours' incubation the hair was found at the anterior end of the primitive streak, the medul-

lary groove lying anterior to the hair. In those of forty hours' incubation the point of insertion lay at the level of the most anterior somites. The results of these experiments, as a whole, indicate that when the blastoderm of an unincubated egg is divided into two halves by a plane passed through the center, perpendicular to the long axis of the later embryo, the half anterior to this plane gives rise to the heart, brain, sense-organs, medulla and fore-gut, and all the rest of the embryo posterior to these organs arises through the activity of the primitive streak region.

The conclusions of Assheton are fully substantiated by the results of the experiments which I made in 1898, although at that time I used embryos in which the primitive streak was already present, or completely formed. I found that an injury made at the anterior end of the primitive streak of the eighteenth hour of incubation, appeared in forty-eight hours in the region just back of the heart, between the first pairs of somites.

Kopsch has recently described (1902) a series of experiments which he made in order to determine whether or not the actual cell material of the primitive streak becomes changed into the embryo. This, he concludes, is true, and he is able to show, through these experiments, that the primitive streak represents definite parts of the embryo. The most anterior end becomes the chordal region of the head, from the middle portion the somites and trunk develop, and from the posterior third the parts of the embryo caudal to the twentieth pair of somites are formed. He believes that the young primitive streak represents these regions in greater concentration. Thus, the entire embryo with the exception of the pre-chordal head area arises directly from the cell material of the young primitive streak.

Kopsch recognizes, as all must who attempt experiments on the early embryo of the chick, the great difficulties arising from the variation in degree of development at a given hour, and also the great danger, in the living egg of failure in locating the exact region that is to be injured. These difficulties may account in part for the difference in the results obtained by Kopsch, and by Assheton and myself.

In the spring of 1902 I made a series of experiments in

which I used Kopsch's method of injuring certain regions by the introduction of electrodes instead of cauterizing with a hot needle. I was not so successful with this method as I have been with the hot needle. I repeated my own experiments in which the anterior end of the primitive streak of eighteen hours had been killed, and obtained the same result as before; the injury appeared after twenty-four to forty-eight hours in the region of the anterior somites. Kopsch did not injure the anterior end of the primitive streak of an embryo younger than twenty-four hours. He found that an injury made at the anterior end of the primitive streak at this time appears after incubation in the brain, greatly disturbing the development of that region. From this he concludes that the anterior end of the primitive streak represents the chordal region of the head. I have found that an injury made in the area pellucida immediately *in front* of the primitive streak of eighteen hours, appears in this region of the head. It seems reasonable, therefore, to conclude that Kopsch has injured the head process instead of the primitive streak, for by the twenty-fourth hour the former is fully developed.

I can not understand how Kopsch is able to locate the anterior end of the primitive streak in a twenty-four-hour embryo, for eggs incubated in the laboratory at Bryn Mawr, and in my own laboratory in Baltimore, at twenty-four hours show the medullary folds, the first pair of somites, and the notochord; moreover, in the living egg it is almost impossible to determine where the primitive streak ends and the head process begins. After the twentieth hour I have found it impossible, unless development has been delayed, to locate the anterior end of the primitive streak.

I have repeated Kopsch's experiment, and have intentionally injured the distal end of the head process. At the end of twenty-four to forty-eight hours the injured region has been found in the brain, in a position corresponding to that indicated by Kopsch as the result of supposed injury to the anterior end of the primitive streak. If Kopsch had experimented on an embryo of the age indicated by his figures for sixteen and a half

hours, I think he would undoubtedly have found that the anterior end of the primitive streak does not, in the later embryo, represent a region beyond the anterior somites, and therefore, does not take part directly in the formation of organs anterior to the heart.

THE WOMAN'S COLLEGE, BALTIMORE, MD.,
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